UNITED STATES DISTRICT COURT
WESTERN DISTRICT OF WISCONSIN

PROMEGA CORPORATION,

Plaintiff,

MAX-PLANCK-GESELLSCHAFT ZUR FORDERUNG DER WISSENSCHAFTEN E.V.,

Case No.: 10-CV-281

Involuntary Plaintiff,

v.

LIFE TECHNOLOGIES CORPORATION, INVITROGEN IP HOLDINGS, INC., and APPLIED BIOSYSTEMS, LLC,

Defendants.

DECLARATION OF DR. RANDALL DIMOND IN SUPPORT OF PLAINTIFF'S MOTION FOR SUMMARY JUDGMENT

I, Dr. Randall Dimond, declare under penalty of perjury pursuant to 28 U.S.C. § 1746 as follows:

1. I make this declaration on behalf of Plaintiff, Promega Corporation ("Promega"), in support of Plaintiff's Motion for Summary Judgment.

2. I have previously supplied an initial expert report (attached hereto as Exhibit A) and a rebuttal expert report (attached hereto as Exhibit B) as part of the above-captioned litigation. These expert reports set forth the information I have relied upon in formulating the opinions set forth in those expert reports, as well as in this Declaration. My qualifications to testify in this action are set forth in my initial expert report.

3. In preparing this report, I reviewed the so-called Tautz Patent (RE37,984) as well as the Promega Patents, i.e. U.S. Patent Nos. 5,843,660 ('660); 6,221,598 ('598); 6,479,235 ('235); and 7,008,771 ('771). I am familiar with the technology utilized in these patents. The Promega Patents disclose and claim methods of simultaneously determining specified DNA markers in a human genome by methods that include multiplex PCR. The primary applications for these methods in the past have been in the fields of forensic analysis, DNA typing, and paternity determination. The nature of each of these fields is briefly discussed below.

A. Nature of the Fields Applicable to Multiplex STR Analysis

4. For the reasons detailed below, it is my opinion that the use of STR multiplex kits in the following applications are not "Forensics and Human Identity" or "Paternity" applications: monitoring bone marrow transplant engraftment; genotyping hydatidiform moles; cancer analysis; determination of fetal sex; archeological and anthropological research; clinical research on inherited genetic and acquired diseases; and confirming that cell cultures or cell lines are uncontaminated, correctly identified, and/or genetically unique (collectively cell line authentication).

5. A useful definition for Forensic Testing is found on the Human Identity Trade Association (HITA) website (http://humanidentity.org):

"Forensic DNA testing serves a number of useful purposes. It can be used to track down criminal suspects who have left behind biological evidence, exonerate individuals who have been falsely accused of committing crimes, identify individuals who have fallen victim to violent crimes or disasters, and connect crimes that share biological

evidence. It can also be used to identify the father of a child conceived through rape or incest."

To summarize, forensic uses are legal in nature and include a) uses to inculpate perpetrators and exclude falsely accused individuals, b) uses to identify victims of a violent crime or mass fatality, or c) identify the father in a criminal paternity case.

6. Forensics involves human identity testing. The fundamental question for human identity testing is: who is this person? The process starts with a sample, the human source of which is unknown, and (if successful) proceeds to the identification of the human from which the sample is derived.

7. That clinical uses are distinct from forensic and paternity uses can be seen from the language of the Promega patents. For example, the '771 Patent notes that the technology has specific uses "in the field of forensic analysis, paternity determination, monitoring of bone marrow transplantation, linkage mapping, and detection of genetic diseases and cancers." ('771 Patent, col. 5, lines 50-53).

8. There is a definition in a 2006 Promega agreement with Applera/ABI for "Forensic and Human Identity Applications" with the following language: "Forensics and Human Identity Applications" means any analysis, based on the measurement of the length of polynucleotide sequence containing a tandem repeat, of human genetic material for (a) use in, or preparation for, legal proceedings, or (b) analysis of biological specimens for identification of individuals." This definition is consistent with the HITA definition for Forensic testing (see paragraph 4 above) in that the testing is for legal proceedings or for the identification of individuals, such as in disasters.

9. The words "analysis . . . for identification of individuals" in the 2006 Promega agreement are consistent with my statement above regarding the fundamental question of human identity testing, i.e. who is this person? There is nothing in this language from the 2006 Promega agreement that suggests to me that "Forensics and Human Identity Applications" includes STR testing for clinical or research purposes.

10. "DNA typing" is a more general term than Forensic or Paternity testing. DNA typing can involve the typing of tissue or cell cultures where the question is not: who is this person? For example, DNA typing by multiplex analysis of STR loci today is routinely done for research purposes and for clinical purposes, e.g. clinical diagnostic, treatment, and clinical research purposes. Clinical diagnostics involves diagnostics in a clinical setting, e.g. a setting in which clinical diagnostic results influence patient treatment. Similarly, clinical research involves research in a clinical setting. It typically involves the use of human subjects or materials directly derived from human subjects. By contrast, research that is not done in a clinical setting typically does not involve living human subjects but encompasses a wide variety of experimental questions and subject material.

11. Starting in the mid-1990s, there has been a growing use of commercial STR kits for clinical purposes, including but not limited to monitoring bone marrow transplantation engraftment, genotyping hydatidiform moles, characterizing and diagnosing cancer, and contamination testing (including cell line authentication). See J. Pfeifer *et al*., The Changing Spectrum of DNA-based Specimen Provenance Testing in Surgical Pathology, *Am. J. Clin. Path*., 135:132 (2011) that utilized AmpFlSTR® Profiler Plus® (attached as Exhibit 1). All of these applications of STR multiplex analysis are

distinct from forensic and paternity applications and are discussed in some detail in the sections below.

a. bone marrow engraftment monitoring

12. The monitoring of bone marrow transplants in human patients is done in a clinical setting, i.e. the transplant is done for the treatment of the patient. The fundamental question involved in monitoring transplant engraftment using STR multiplexing is not: who is this person? It is not for the purpose of human identity because, after all, the identity of the donor and recipient in the transplant is known. Rather, it is to monitor the engraftment of the transplant, i.e. whether the donor bone marrow cells are growing and contributing to the circulating blood cells of the recipient. Any residual recipient circulating blood cells are also monitored for a variety of purposes including detection of recurrence of disease. Thus, this clinical field is clearly distinct from the forensic field.

13. Following bone marrow transplantation, the recipient may produce their own (host) cells as well as donor blood cells. This is called "chimerism," i.e. a mixture of cells originating from two individuals. Chimerism analysis provides quantitative information about the transplant and can serve as a prognostic factor. Commercial multiplex STR kits and their associated protocols are carefully designed so that the relative amount of amplified DNA for each STR allele is proportional to the amount of genomic DNA containing that allele in the sample being analyzed. Thus, comparing the amount of amplified STR alleles from donor versus host provides an indication of the proportion of blood cells contributed by each source. Repetitive testing over time

provides an indication as to whether the proportion of blood cells from the donor and host are changing, which has treatment and prognostic value.

14. As noted in Liang et al., there are instances where "decisions to alter treatment are based on major shifts in the degree of chimerism . . .". Liang *et al*., *J. Mol. Diag*. 10:142-6 (2008) at p. 145 (attached hereto as Exhibit 2). In some cases where analysis of chimerism indicates a relapse risk, relapse can be prevented by changing therapy, e.g. withdrawing immune suppression and/or by administering infusions of donor cells (such as donor lymphocytes). See Pulsipher *et al*., *Biol. Blood Marrow Transplant*, 15:62-71 (2009) at p. 65 (attached as Exhibit 3).

15. Thus, in the clinical field of bone marrow transplantation, multiplex STR analysis to monitor bone marrow engraftment has become an important tool for the physician to increase the chances of transplant success in treating malignancies. While there are other tests that might be used, "the overwhelming majority of laboratories" continue to use STR multiplexing in this manner. Liang *et al*., *J. Mol. Diag*. 10:142-6 (2008) at p. 142 (attached hereto as Exhibit 2) (see introduction). Liang *et al*. used the AmpFlSTR® Profiler® kit for monitoring of bone marrow engraftment.

16. Chimerism analysis is generally performed serially. The presence of recipient cells following transplantation (particularly a non-ablative transplant) may not indicate residual disease or relapse because normal/non-malignant recipient cells may be produced post-transplant. Therefore, serial analyses are performed to monitor for an increase in recipient cells, which is associated with the reappearance of the underlying disease. See Pulsipher *et al*., *Biol. Blood Marrow Transplant*, 15:62-71 (2009) at p. 65 (attached as Exhibit 3).

17. The time interval between a change in chimerism and relapse can be very short. Therefore, serial analysis of chimerism by STR-PCR needs to be done frequently during the first 100 to 200 days after transplantation, when most relapses occur. See Pulsipher *et al*., *Biol. Blood Marrow Transplant*, 15:62-71 (2009) at p. 66 (attached as Exhibit 3). Some groups emphasize repeated testing in the first month post-transplant followed by increased time periods between testing during the first year and annual testing thereafter. See Kristt *et al*., Assessing Quantitative Chimerism Longitudinally, *Bone Marrow Transplant*, 39:255-68 (2007) at p. 266 (attached as Exhibit 4). Where other markers are not available, sequential analysis of the percent of chimerism "may offer the only evidence on which sub-clinical relapse of disease can be assessed . . . " Kristt *et al*., at page 261.

18. With regard to the field of parentage testing, the HITA website once again has a useful definition:

"Parentage testing is the examination and comparison of an alleged parent's and a child's genetic profiles to determine whether the individuals are biologically related as parent and child. There are two types of parentage tests: paternity tests and maternity tests."

Thus, where one is not seeking to determine the parent of a child by comparing BOTH profiles, it would not fall in the definition.

19. Certainly, monitoring bone marrow engraftment from a transplant between adults would not fall within the above-noted parentage testing definition. Moreover, even where a child and a parent are involved in the bone marrow transplant, the testing would not be "to determine whether the individuals are biologically related."

20. Thus, monitoring bone marrow engraftment using multiplex STR analysis is outside the field of parentage testing. The patient material is simply being analyzed to ensure the graft has taken hold in the recipient (and to what extent).

21. There is a definition in the 2006 Promega agreement with Applera/ABI for "Paternity Applications" with the following language: "Paternity Applications" means any analysis, based on the measurement of the length of a polynucleotide sequence containing a tandem repeat, of human genetic material for parentage determination, except in cases of sexual assault investigations." This application is for legal purposes in determining parental rights and obligations or for resolving immigration issues. I find this definition consistent with the HITA website definition of parentage testing, since the point of paternity testing, as defined in the agreement, is to determine one or more of the parents. There is nothing in this language from the 2006 Promega agreement that suggests to me that "Paternity Applications" includes STR testing for clinical or research purposes.

22. The AmpFISTR® Profiler Plus® PCR Amplification Kit was used to determine T cell chimerism at the Virginia Commonwealth University Medical Center (Epp *et al*., *J. Mol. Diag.* 9(3): 665-6 (2007)) (Exhibit 5). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

23. The AmpFlSTR® Profiler Plus® and COfiler® PCR Amplification Kits were used for monitoring chimerism using fluorescent multiplex PCR of STRs and compared the limit of detection and heterogeneity of STR loci by Genzyme Genetics, Westborough MA (Hire *et al*., "Bone Marrow Engraftment Chimerism Monitoring by

STR Analysis" 2004) (Exhibit 6). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

24. The AmpFlSTR® Profiler® PCR amplification kit was evaluated as a clinical test to determine chimerism or reappearance of recipient blood cells during bone marrow engraftment monitoring by John Hopkins University (Liang *et al. J. Mol. Diag*. 10: 142-6 (2008)) (Exhibit 2). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

25. The AmpFISTR® Profiler Plus® kit was tested with an algorithm to reduce the impact of stutter on bone marrow transplant chimerism monitoring by the University of Minnesota (Thyagarajan *et al*. *J Clin Lab Anal* 23:308-11 (2009)) (Exhibit 7). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

26. Post-transplant lymphoproliferative disorder (PTLD) is a major complication of organ transplantation. Most PTLDs following solid organ transplant are of recipient origin. PTLDs are mostly of donor origin following bone marrow or stem cell transplantation. The AmpFlSTR® Identifiler® PCR amplification kit was used to determine that tumor cells from a particular patient were of donor origin by the University of Medicine & Dentistry of New Jersey (Zhang, H. *et al*. *Blood* (ASH Annual Meeting Abstracts) 114 Abstract 4305 (2009)) (Exhibit 8). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

27. The AmpFISTR® Profiler Plus® Kit was used to determine stem cell transplantation success in either the traditional ablative or the newer non-myeloablative methods of reconstitution of the patient's bone marrow with the donor's stem cells in order to establish a complete donor chimera by the City of Hope National Medical Center (Senitzer and Giadulis, Scientific Communication: American Society For Histocompatibility and Immunogenetics (ASHI) Quarterly Second Quarter 2001) (Exhibit 9). Stem cells are the types of cells that engraft during bone marrow transplantation. For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

28. The AmpFlSTR® Profiler® Kit was routinely used to evaluate polymorphic differences between the patient and donor that allows for quantification of chimerism after bone marrow transplantation by Johns Hopkins University (Murphy, K. M. *et al*., *J. Mol. Diag*. 9:408-13 (2007)) (Exhibit 10). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

29. The ARUP clinical diagnostic laboratory headquartered in Salt Lake City, UT offers a variety of STR clinical diagnostic tests utilizing the Identifiler® Kit for bone marrow transplantation and chimerism determination. These include: Chimerism Donor (Exhibit 11); Chimerism post-transplant sorted cells (Exhibit 12); Chimerism post-transplant (Exhibit 13); and Chimerism Pre-Transplant Recipient (Exhibit 14). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

30. The Molecular Diagnostic Laboratory of the Barnes Jewish Hospital associated with the Washington University School of Medicine utilizes the Profiler Plus® Kit in a clinical diagnostic assay they offer for monitoring bone marrow engraftment. (Exhibit 15). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

31. Transplant engraftment monitoring has been expanded to types of cellular transplants not involving bone marrow or hematopoietic stem cells. A review chapter by Fisher & Mas in *Hepatocyte Transplantation* 481, Chapter 9, pages 97-105 (2009) (selected pages of which are attached as Exhibit 16) provides directions for the use of the Profiler Plus® Kit for monitoring of liver engraftment of hepatocyte infusions for correction of liver deficiencies. For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

b. genotyping hydatidiform moles and managing GTD

32. Multiplex STR analysis can be very useful in other clinical situations, such as genotyping hydatidiform moles. See K. Murphy *et al*., "Molecular Genotyping of Hydatidiform Moles," *J. Mol. Diag*. 11:598-605 (2009) (attached hereto as Exhibit 17). Unfortunately, distinguishing between different types of moles based on morphology is difficult, with groups reporting "poor interobserver and intraobserver reproducibility." See F. Lipata *et al*., "Precise DNA Genotyping Diagnosis of Hydatidiform Mole," *Obstetrics & Gynecol.* 115(4): 784-94 (2010) (introduction section) (attached hereto as

Exhibit 18). The commercially available Applied Biosystems (AB) multiplex STR analysis kits have been found to be "applicable to routine practice for classifying molar specimens" See K. Murphy *et al*., Molecular Genotyping of Hydatidiform Moles, *J. Mol. Diag*. 11:598-605 (2009) at p. 604 (attached hereto as Exhibit 17).

33. Distinction of hydatidiform moles from non-molar specimens and the sub-classification of hydatidiform moles as complete ("CHM") or partial ("PHM"), are important for both clinical practice and investigational studies. Accurate classification is critical to ascertaining the woman's risk of persistent gestational trophoblastic disease (GTD) and determining the appropriate nature and duration of clinical follow-up care. Both under-diagnosis and over-diagnosis of hydatidiform moles can result in faulty estimation of the risk of persistent GTD and improper clinical management.

34. The fundamental question involved in classifying molar specimens using multiplex STR analysis is not: who is this person? It is not for the purpose of human identity because, after all, the identity of the person with the potential molar pregnancy is known. It is also not for the purpose of determining the father. Rather, it is done to determine whether mother is at risk for more serious disease.

35. The AmpFlSTR® Profiler® Kit was used for the molecular genotyping of hydatidiform moles from stored historical samples to determine their classification by Johns Hopkins University (Murphy et al., *J. Mol. Diag*. 11:598-605 (2009)) (Exhibit 17). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

36. The AmpFlSTR® Identifiler® PCR Amplification Kit was used to diagnose and subtype hydatidiform moles by Yale University (Lipata et al., *Obstetrics & Gynecol*

115(4): 784-94 (2010)) (Exhibit 18). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

37. The AmpFlSTR® Identifiler® PCR Amplification Kit was used to demonstrate the applicability of DNA genotyping as a molecular approach for the diagnosis, and subtyping of molar pregnancy in daily clinical practice by Yale University (Bifulco et al., *Am J Surg Pathol* 32:445–451 (2008)) (Exhibit 19). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

38. Choriocarcinoma is a malignant, trophoblastic and aggressive form of cancer. It often arises in patients with a previous history of hydatidiform moles. The AmpFlSTR® Profiler Plus® kit was evaluated for diagnosis of gestational and non-gestational choriocarcinomas and differentiation from both benign and malignant tissues of other types including partial and complete hydatidiform moles. Samples had been preserved in paraffin blocks and were separated by microdissection at the Henry Ford Hospital (Cankovic *et al*. *Gynecol Oncol* 103:614-7 (2006)) (Exhibit 20). For the reasons detailed above, it is my opinion that the use of the Profiler Plus® STR kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

39. Gestational Trophoblastic Neoplasm (GTN) is a group of neoplastic diseases composed of choriocarcinomas, placental site trophoblastic tumors, and epithelioid trophoblastic tumors, all of which are derived from fetal trophoblastic tissue. The AmpFlSTR® Profiler® kit was used for sex determination of GTN samples by Johns Hopkins University (Yap et al., *J. Oncology* Volume 2010, Article ID 364508) (Exhibit

21). The majority of samples lacked Y chromosomes suggesting that they arose from earlier complete hydatidiform molar pregnancies. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

40. The AmpFISTR® Profiler Plus® and COfiler® PCR amplification kits were used to determine the zygosity of a hydatidiform mole and to rule out maternal cell contamination thereby elucidating the diagnosis of a complete hydatidiform mole and a co-existing normal fetus by Genzyme Genetics, Westborough, MA (Faulkner *et al*. "Utility of Molecular Zygosity Analysis to Determine the Genetic Composition of a Twin Pregnancy with an Apparent Hydatidiform Mole") (Exhibit 22). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

41. The AmpFlSTR® Profiler® Kit was used to diagnose hydatidiform moles to distinguish partial (PHM) and complete (CHM) types from one another as well as from non-molar abortuses (NMA) by Johns Hopkins University (Hafez *et al*., J. Mol. Diag. 9: 691 (2007) (Abstract)) (Exhibit 23). For the reasons detailed above, it is my opinion that the use of STR multiplex kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

42. The AmpFlSTR® Profiler® and AmpFlSTR® Identifiler® kits were used to perform molecular genotyping, which determined that a sample from a spontaneous abortion exhibited biparental diploidy with trisomies of chromosomes 7, 13, and 20, all of paternal origin. The sample demonstrated abnormal villous morphology indicating that this sample with excess paternal genetic material had some characteristics of a partial

hydatidiform mole. This diagnosis was reported by Johns Hopkins University (Kirby-Norris *et al*., *J Mol Diag* 1: 620 (2009) (Abstract)) (Exhibit 24) and (Kirby-Norris *et al*., *J Mol Diag* 12:525-9 (2010) (Abstract)) (Exhibit 25). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

43. The ARUP clinical diagnostic laboratory headquartered in Salt Lake City, UT offers a clinical diagnostic test utilizing the Identifiler® Kit to detect molar pregnancies called: Molar Pregnancy, 16 DNA Markers, 0051755. (Exhibit 26). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

c. **cancer analysis**

44. The analysis of STRs is very useful for understanding a variety of cancers in addition to choriocarcinoma. The AmpFlSTR® Identifiler® kit was used to analyze genetic instability in a variety of different cancers by detecting allelic imbalance. In a set of 239 tumor samples, 67% contained two or more STR loci demonstrating allelic instability. This work was performed at the University of New Mexico (Heaphy *et al*., *J. Mol. Diag*. 9(2):266-271 (2007)) (Exhibit 27). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

45. The AmpFlSTR® Identifiler® PCR Amplification Kit was used to demonstrate that Exaggerated Placental Site reaction, a non-neoplastic condition, is not a

precursor to Placental Site Trophoblastic Tumor (PSTT), a true neoplastic proliferation of intermediate trophoblasts in clinical research performed by the Harvard and Yale University Medical Schools (Jorge and Hui, *Int. J. Gynecol. Path.* 27:562-7 (2008)) (Exhibit 28). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

46. A clinical research study published by Carvalho *et al*., in *DNA and Cell Biol.* 29(1):3-7 (2010) (Exhibit 29) discovered that some of the Y-chromosome STR alleles analyzed by the Yfiler® Kit were associated with the occurrence of prostate cancer. They propose that the Yfiler® Kit can be used as a clinical diagnostic assay for prostate cancer risk. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

d. cell culture authentication

47. Multiplex STR analysis is also useful to assist in confirming that cell lines thought to be independently derived are in fact genetically unique. This is useful in a variety of settings, including clinical research and basic research. In one study, multiplex STR analysis with commercially available AB kits was performed on 40 reported thyroid cancer-derived cell lines, only to reveal that a) many were not unique, and b) some were not even of thyroid origin. See R. Schweppe *et al*., "Deoxyribonucleic Acid Profiling Analysis of 40 Human Thyroid Cancer Cell Lines Reveals Cross-Contamination Resulting in Cell Line Redundancy and Misidentification," *J. Clin. Endocrinol. Metab*.

93:4331-41 (2008) (attached hereto as Exhibit 30) (see Abstract). As noted in this report (p. 4332), the NIH now recognizes this problem and requires a commitment to use of cell authentication with all grant applications. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

48. Cell line authentication by multiplex STR analysis is a type of tissue typing. In general, tissue typing is the analysis of various markers capable of characterizing the similarities and differences between tissues from different individuals. Historically, the term has been frequently used in conjunction with organ transplantation where various immunological markers are typed to match tissues to avoid transplant rejection. More recently, STR typing and other methods of DNA typing have become methods of tissue typing of increasing utilization in research and clinical diagnostics as reflected in the discussion above. Use of multiplex STR analysis for cell line authentication is not human identity testing because the purpose for testing the cell line is not for identification of the person from whom the cell line was established. Rather, its purpose is to confirm that the intended cell line is actually the one involved in the research and is a measure of the genetic integrity of the cell line. In cases where new cell lines are being established or studied, multiplex STR analysis is used to establish that the new cell lines are unique.

49. Cell line authentication is not a problem related to forensic or paternity testing. It is not a problem for human identity testing since the fundamental question is not: who is this person? It has become an essential research application because use of an incorrect cell line can invalidate a researcher's published data.

50. The AmpFlSTR® Identifier® PCR Amplification Kit was used to verify cell line identities by the University of Arizona (Shaw *et al*., *J Pharmacol Exp Therapeut* 331(2):636-647 (2009)) (Exhibit 31). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

51. The AmpFlSTR® Identifiler® PCR Amplification Kit was used to test and authenticate thyroid cell lines used to explore the mechanism of gene expression changes in thyroid tumor samples by the University of Pittsburgh (Zuo *et al*., Cancer Research 70(4); 1389–97 (2010)) (Exhibit 32). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

52. The AmpFlSTR® Identifiler® Kit was used to validate the MDA- MB231 breast cancer cell line by the M.D. Anderson Cancer Center (Iadevaia et al., Cancer Resesarch 70(17):OF1–11 (2010)) (Exhibit 33). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

53. The AMPFlSTR® Identifiler® Kit was used to distinguish human embryonic stem cell lines, even after genetic modification and in different culture conditions by Rutgers University (Cardoso et al., Poster) (Exhibit 34). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

54. The AMPFlSTR® Identifiler® Kit was and is used to provide human cell line authentication services by Biosynthesis, Inc. (http://www.biosyn.com/

celllinetesting.aspx) (an excerpt from this website is attached as Exhibit 35). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

55. The AMPFlSTR® Identifiler® Kit was and is used to provide DNA profiling services, including cell line authentication, by the University of Colorado (http://DNASequencingCore.ucdenver.edu) (an excerpt from this website is attached as Exhibit 36). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

56. The AmpFlSTR® Identifiler® PCR Amplification Kit was and is used to provide cell line authentication services by Johns Hopkins University (http://faf.grcf.jhmi.edu/str.html) (an excerpt from this website is attached as Exhibit 37). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

57. The AmpFlSTR® Identifiler® PCR Amplification Kit was and is used to provide cell line authentication services by The University of Arizona (http://uagc.arl.arizona.edu/index.php/dnacell-fingerprinting.html) (an excerpt from this website is attached as Exhibit 38). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

58. The AmpFlSTR® Identifiler® PCR Amplification Kit was and is used to provide cell line authentication services by The University Of Pittsburg (University of Pittsburgh Cancer Institute Cell Culture and Cytogenetics Facility website: http://www.upci.upmc.edu/cytogen/serv.cfm) (an excerpt from this website is attached as Exhibit 39). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

59. The AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) was and is used to perform cell line authentication by determining the unique genetic signature of each of several new cell lines by Rutgers University (Moore *et al*., Stem Cell Research 4:92–106 (2010)) (Exhibit 40). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

60. The AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) was and is used to provide cell line authentication services by SeqWright DNA Technology Services (http://www.seqwright.com/researchservices/humancellline.html) (an excerpt from this website is attached as Exhibit 41). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

61. The AmpFlSTR® Identifiler® Kit was and is used to perform cell line authentication for genetic identification of cell lines and xenografts by the Children's Oncology Group Cell Culture and Xenograft Repository

(http://www.cogcell.org/clid.shtml) (an excerpt from this website is attached as Exhibit 42). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

62. The AmpFlSTR® Identifiler® Kit was and is used to perform cell line authentications by the University of Southern California (Cabral *et al*., Proc Am Assoc Cancer Res 48:96 (2007)) (Exhibit 43). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

63. A similar application involves the use of STR analysis to confirm the identification of biopsy specimens in clinical research and clinical diagnostics. In clinical research reported by Dong *et al*., from the Emory University School of Medicine in *Human Mol. Genet.* 17(7):1031-42 (2008) (Exhibit 44), the Identifiler® Kit was used to confirm that paired samples of prostate cancer tissue and normal tissue believed to have been taken from the same patient were in fact from the same patient. These confirmation tests are used to rule out contamination or misidentification of surgical biopsy samples in the clinical diagnostic laboratory are sometimes referred to as specimen provenance testing. The purpose of the test is not to identify an individual but to confirm the identity and source of the biopsy sample. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

e. determination of fetal sex

64. For a number of diseases where the causative gene is on the X-chromosome (X-linked diseases) determination of the sex of the fetus at very early stages of development is of great importance. Female fetuses have two X chromosomes and are less likely to be affected by recessive X-linked disease genes. The parental relationship of the fetus is not important in these cases - only its sex. AmpFlSTR® Identifiler® and Yfiler® Kits were used to develop a new diagnostic test for fetal sex utilizing fetal DNA present in maternal plasma. (Wagner *et al*., *Prenatal Diagnosis* 28:412-6 (2008))(Exhibit 45). The Yfiler® Kit was found to provide a more reliable assay. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits for clinical research or clinical diagnosis in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

f. archeological and anthropological research

65. The Y-chromosome is particularly useful in studies of ancient peoples and their geographical migration at different periods of time in history. Most of the Y-chromosome contains DNA sequences that have no similar sequences (homologs) on other chromosomes. Also, only a single copy of the Y-chromosome at most exists in any cell. Therefore, recombination is greatly suppressed and large segments of the Y-chromosome DNA sequence are passed from generation to generation unchanged. Analysis of STR alleles at different Y-chromosome loci demonstrate patterns of co-inheritance that allow these relatively unchanging segments of DNA to be identified.

These patterns of inheritance are referred to as haplotypes. The slow rate of change of Y-haplotypes allows the study of the geographical movement of men carrying these Y-chromosomes over long periods of history.

66. The Yfiler® Kit was used to study the relationship between different Tamil castes in India compared to other populations around the world. (Balamurugan *et al*., Legal Medicine 12:265-9 (2010)(Exhibit 46). There was no determination of paternity in this study and no attempt to identify any person as all individuals involved were volunteers known to the researchers. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

g. clinical research on inherited genetic and acquired diseases

67. The Yfiler® Kit was used to determine the number of Y-haplotypes present in the Mendoza population of Argentina in a research study published by Marin & Furfuo in *Forensic Sci. Int'l.: Genetics* 4:e89-93 (2010)(Exhibit 47). They studied the impact of historical European migration on the male population of this group of people. A similar study using the Yfiler® Kit concerning the population of South-East Romania was reported by Stanciu *et al*. in *Legal Medicine* 12:259-64 (2010) (Exhibit 48). A similar study using the Yfiler® Kit concerning the population of Yakutia was reported by Theves *et al*. in *Forensic Sci. Int. Genet.* (2010) (Exhibit 49). A similar study using the Yfiler® Kit concerning the population of the El Beni Department of North Bolivia was reported by Tirado *et al*. in *Legal Medicine* 11:101-3 (2009) (Exhibit 50). A similar study using the Yfiler® Kit concerning the population of South Poland was reported by

Wolanska-Nowak *et al*. in *Forensic Sci. Int. Genet.* 4:e43-4 (2009) (Exhibit 51). Vermeulen *et al*. in *Forensic Sci Int. Genet* 3:205-13 (2009) (Exhibit 52) studied the historical relatedness of men from populations around the world using the Yfiler® Kit. Paternity relationships were not studied and no individuals were identified as a result of the studies. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

68. Twins are often utilized in studies of the inheritance of genetic risk factors for various human genetic diseases. Zygosity or the relationship between the twins is studied to confirm that they are in fact twins and to differentiate identical from fraternal twins.

69. A study of genetic and environmental influences on increased plasma homocysteine utilized the Profiler Plus® Kit to determine zygosity. (Bathum *et al*, Clin. Chem. 53(5):971-9 (2007)) (Exhibit 53). The kit was not used to determine parentage or to identify any individual. For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within "Forensics and Human Identity" or "Paternity" applications.

70. The Profiler Plus® Kit was instrumental in a clinical research study that indicated a role for chimerism in the early stages of development of thyroid autoimmunity in twins. The kit was used to confirm and analyze the nature of the zygosity of a large number of twins that participated in this study. (Brix *et al*., J Clin Endocrinol. Metab. 94(11)4439-43 (2009)) (Exhibit 54). There was no determination of parentage or identification of individuals in this study. For the reasons detailed above, it

is my opinion that the use of multiplex STR amplification kits in this manner does not fall within “Forensics and Human Identity” or “Paternity” applications.

71. The Profiler® Kit was used to analyze twin zygosity in a clinical research study entitled, “Interactions Between Secondhand Smoke and Genes That Affect Cystic Fibrosis Lung Disease,” by Collaco *et al*., in *JAMA* 299(4):417-24 (2008) (Exhibit 55). For the reasons detailed above, it is my opinion that the use of multiplex STR amplification kits in this manner does not fall within “Forensics and Human Identity” or “Paternity” applications.

B. Brief Review of the STR Technology At Issue

72. One can target and identify certain locations or "loci" on the chromosomes which are polymorphic within a population, i.e., loci that vary from individual to individual within the population. These loci are useful as identifiers.

73. The more it varies from individual to individual, the more "polymorphic" the locus is said to be. No one locus alone, however, will positively identify an individual to a statistically significant degree, since no one locus is unique to each individual within any given population. Consequently, for purposes of forensic and paternity determinations, the identification of multiple polymorphic loci is necessary.

74. One can use enough loci with sufficient polymorphic characteristics such that the identification is so statistically significant that the result cannot be reasonably disputed, i.e., the individual is identified beyond any reasonable doubt. For example, using Promega's genetic identity products, one can identify an individual's DNA with a

power of discrimination exceeding 1 in 100,000,000,000. However, where the DNA sample is of poor quality, the power of discrimination can be less than this.

75. STRs are loci found within genomic DNA that have a number of short repetitive nucleotide sequences. Different authors have slightly different definitions with regard to the repeat length that is considered an STR.

76. The DNA sequences at a particular STR locus (singular form of loci) within a given population will exhibit a variable number of these repeat sequences. For some individuals within a given population the sequence will repeat 7 times, for others 8 times, for others 4 and so on. It is this variation in the number of repeats at a particular locus that is responsible for the polymorphism, which permits scientists to genetically distinguish one individual from another.

77. The particular genetic information or base sequence associated with a segment of DNA at a particular STR locus in one individual is called an "allele." The alleles are numbered in accordance with the number of repeated nucleotide motifs (the "motif" is the specific repeated nucleotide sequence, e.g., AATG, of the short tandem repeat).

78. PCR is one method of amplifying. There are several steps in the PCR process. First, the "double stranded" or two strands of genomic DNA are separated or "denatured," thereby forming "single stranded" DNA. This denaturation step is done by heating the DNA to a certain temperature, which is sufficient to cause the two strands to separate. Second, a pair of PCR "primers" is introduced and allowed to hybridize or pair with the single stranded DNA. "Hybridization" occurs when the PCR primers "anneal" or join to a single strand of the DNA. This hybridization occurs in accordance with the

nucleotide pairing rules (e.g. A with T, etc.) noted above, i.e., at a point on the single stranded DNA where the PCR primer sequence is complementary to the genomic nucleotide sequence. Referring to the two opposing primers as the "forward" or "reverse" PCR primer differentiates each primer in the pair.

79. The PCR primers hybridize at points on the genomic DNA that are adjacent to, or "flank," the actual STR locus. These "flanking regions" are used as the point of hybridization because they are not polymorphic, i.e., they contain the same sequence of nucleotides for all individuals within a given population even though the number of repeats contained in the STR locus between the flanking regions varies from individual to individual. This ensures that all alleles in all individuals will be amplified.

80. Amplifying the alleles present at a single STR locus is commonly referred to as a "monoplex" reaction. Amplifying multiple STR locus simultaneously is a “multiplex” reaction.

81. Multiplex PCR is key to realizing the advantages of STRs for determining genetic identity. Often, one must be able to analyze multiple STRs from a relatively small amount of sample. That could only be accomplished if it were possible to multiplex the amplification of STR alleles in such a way that you could still determine all of the alleles present at each locus. The technology of the Promega Patents accomplished that result.

82. The amplified alleles from one DNA sample can be compared to the amplified alleles of a second DNA sample by, for example, running the two samples side-by-side on the gel. One can then determine whether or not the alleles in two samples are consistent with the samples originating from the same individual. Additionally a “size marker” or “allelic ladder” is often run concurrently with the sample either mixed with

the sample (size marker) or in another lane of the gel (allelic ladder). The size standard allows precise determination of allele size and reduces variability between lanes or CE capillaries. By comparing the size of alleles amplified from the DNA sample to the size of the alleles in the allelic ladder one can determine precisely which alleles are present in the DNA sample.

C. Terms Used In The Tautz Patent

83. The '984 Tautz Patent refers to "direct repeats" and "irregularly direct repeats" (i.e. cryptically simple repeats). A direct repeat is one that is in the same orientation as the index sequence. For example, CAG…..CAG is a direct repeat; CAG…GAC is an inverted repeat. What makes a direct repeat a simple sequence is that it is a tandem repeat. A tandem repeat is a repeat with no intervening nucleotides. For example, CAGCAG is a direct tandem CAG repeat, while CAGTCAG is a non-tandem or irregular direct repeat (i.e. cryptically simple repeat).

84. The underlined portion with arrows underneath in Fig 2 of the Tautz Patent is a simple repeat of 8 base pairs GCTAACTA. There are two tandem copies so it is a simple repeat.

D. CODIS STR Loci

85. CODIS is a database system that stores information about the alleles present in individuals that have been STR typed by the FBI or State crime laboratories. The database stores information on particular STR loci including those that make up the CODIS standard 13 loci. For specific CODIS STR loci (Exhibit 58) one can look on the

web to determine the repeat structure (see Exhibit 59a through 59n). For example, http://www.cstl.nist.gov/strbase/str_D5S818.htm shows the repeat structure for D5S818, which is a CODIS STR locus (Exhibit 59d). When one looks at the column listing the "Repeat Structure" one sees that all of the alleles with sequences shown are simple repeats. By contrast, when one looks at http://www.cstl.nist.gov/strbase/str_D21S11.htm, which is CODIS STR, D21S11, one finds all of the alleles at this locus are cryptically simple repeats of various types (Exhibit 59m).

E. ABI/LifeTech Kits and Protocols

ABI/LT Identifiler® Kit

86. The Product Insert for the ABI Identifiler® PCR Amplification Kit (Exhibit 60) lists "PCR Reaction Mix" (which contains the triphosphates in a PCR buffer), primer sets, AmpliTaq Gold® DNA Polymerase, and "Control DNA 9947A" among the components of the kit. Clearly these are reagents for performing a PCR amplification reaction. While the control DNA is provided, the test DNA is not in the kit and is provided by the particular lab where the kit will be used. The primers will anneal to this test DNA as part of the PCR reaction. The kits are indicated as able to "amplify and type" in the context of an ABI PRISM machine. *AmpFlSTR® Identifiler® PCR Amplification Kit, Product P/N 4322288 Insert P/N 4322638 REV G*.

87. The Product Insert for AB Identifiler® Kit (Exhibit 60) indicates that a certain amount of "input sample DNA" is needed for good results: "The recommended range of input sample DNA is approximately 0.5-1.25 ng. At Applied Biosystems, the kit components have been used successfully to type samples containing less than 0.5 ng of

human DNA." *AmpFlSTR® Identifiler® PCR Amplification Kit, Product P/N 4322288 Insert P/N 4322638 REV G* [underlining added].

88. The User's Manual for the AB Identifiler® Kit (Exhibit 61) illustrates the point that samples that possess low amounts of template DNA (i.e. < 0.1ng) are subject to allele drop-out. *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* [Figure 4-12].

89. The User Manual for the AB Identifiler® Kit (Exhibit 61) indicates the kit permits one to multiplex (i.e. co-amplify) multiple loci in a single amplification using the "polymerase chain reaction" (PCR): "By adding an additional dye, more loci can be multiplexed in a single PCR amplification as compared to the previous 4-dye system." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* pg 1-2 [underlining added].

90. The User Manual for the AB Identifiler® Kit (Exhibit 61) discusses the degree of amplification of each locus within a "co-amplified" system. *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* pg 4-34.

91. The User Manual for the AB Identifiler® Kit (Exhibit 61) makes the distinction between amplifying each locus alone and the situation where the loci are "co-amplified with the *AmpFlSTR* Identifiler kit." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* pg 4-36 and pg 4-37.

92. The Product Insert for the Identifier® Kit (Exhibit 60) indicates that the kit provides primers that are covalently labeled: "1 tube containing locus specific 6FAM™, VIC®, NED™, and PET® dye-labeled and unlabeled primers in buffer that amplify the

STR loci CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA, and the gender marker, Amelogenin." *AmpFlSTR® Identifiler® PCR Amplification Kit Product P/N 4322288 Insert P/N 4322638 REV G* [underlining added]. All of these STR loci contain tetranucleotide or 4 base pair repeat regions.

93. The User's Manual for the Identifiler® Kit (Exhibit 61) indicates that the dyes are fluorescent labels: "The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFlSTR Identifiler Primer Set from light when not in use. Amplified DNA, AmpFlSTR® Identifiler™ Allelic Ladder and GeneScan™-500 LIZ™ Size Standard should also be protected from light." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* pg 1-7 [underlining added].

94. The User's Manual for the Identifiler® Kit (Exhibit 61) indicates that the kit provides a means to detect separated alleles using fluorescence detection: "The Identifiler kit uses a five-dye fluorescent system for automated DNA fragment analysis." The PCR products are "electrophoretically separated" and "detected" on the ABI PRISM instrument. *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems, pg 1-2 and 3-1. (underlining added).*

95. The Product Insert for the Identifier® Kit (Exhibit 60) is identified as providing an allelic ladder comprising amplified alleles that are covalently labeled: "1 tube of AmpFlSTR® Identifiler® Allelic Ladder containing the following amplified alleles. 6-FAM™ dye (blue): D8S1179 alleles 8-19; D21S11 alleles 24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38; D7S820

alleles 6-15; CSF1PO alleles 6-15. VIC® dye (green): D3S1358 alleles 12-19; TH01 alleles 4-9, 9.3, 10, 11, 13.3; D13S317 alleles 8-15; D16S539 alleles 5, 8-15; D2S1338 alleles 15-28. NED™ dye (yellow): D19S433 alleles 9-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2; vWA alleles 11-24; TPOX alleles 6-13; D18S51 alleles 7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27. PET® dye (red): Amelogenin alleles X and Y; D5S818 alleles 7-16; FGA alleles 17-26, 26.2, 27-30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2." *AmpFlSTR® Identifiler® PCR Amplification Kit Product P/N 4322288 Insert P/N 4322638 REV G* .

96. The User Manual for the AB Identifiler® Kit (Exhibit 61) indicates the components permit "accurate characterization of the alleles amplified," i.e. permitting one to determine the alleles present: "The AmpFlSTR Identifiler® Allelic Ladder was developed by Applied Biosystems for accurate characterization of the alleles amplified by the AmpFlSTR Identifiler® kit. The AmpFlSTR Identifiler® Allelic Ladder contains the majority of alleles reported for the 15 loci." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems, pg 3-32* [underlining added].

97. The User Manual for the AB Identifiler® Kit (Exhibit 61) indicates that the AB system permits detecting and evaluating amplified alleles: "A ±0.5-bp window allows for the detection and correct assignment of alleles. An allele that sizes only one base pair different from an allele in the allelic ladder will not be incorrectly typed and will be identified as off-ladder." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems, pg 4-8* [underlining added].

98. The Identifiler® Kit is identified in the User Manual (Exhibit 61) as containing sufficient reagents so as to permit many PCR amplifications: "The AmpFlSTR Identifiler kit contains sufficient quantities of the following reagents and the appropriate licenses to perform 200 25-µL amplifications …" *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems* pg 1-7 [underlining added].

99. The User's Manual for the Identifiler® Kit (Exhibit 61) indicates that the control DNA can be amplified with the primers to generate many copies of the STR "alleles." *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems (Figure3-6).*

100. The User's Manual for the Identifiler® Kit (Exhibit 61) illustrates results obtained when one has a sample with a mixture of DNA from two individuals. Figure 4-16 provides illustrative results when two samples are mixed at different ratios. There is a discussion of using quantitative analysis on these mixtures to determine which alleles are from the minor DNA contributor on page 4-41. Basically, the instrument quantitates the peak heights for each of the amplified alleles, allowing for a comparison of the relative amount of DNA corresponding to each allele. The amount of amplified DNA for an allele is an approximate indicator of the amount of sample DNA containing that allele. *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual, Copyright 2006, 2010 Applied Biosystems.*

101. The Product Insert for the Identifiler® Kit (Exhibit 60) identifies a size standard as "Required Materials – Not Included" and describes the size standard as follows:

GeneScan™-500 LIZ® Size Standard 4322682 2 tubes each containing 200 µL of size standard. Loading buffer is included as a separate tube. GeneScan™-500 LIZ® Size Standard (not GeneScan™-350 ROX™ or GeneScan™-500 ROX™ Size Standards) must be used with the AmpFlSTR® Identifiler® kit.

AmpFlSTR® Identifiler® PCR Amplification Kit Product P/N 4322288 Insert P/N 4322638 REV G [underlining added].

ABI/LT Profiler® Kit

102. The Product Insert for the AB Profiler® PCR Amplification Kit (Exhibit 62) lists "PCR Reaction Mix" (which contains the triphosphates in a PCR buffer), primer sets, AmpliTaq Gold® DNA Polymerase, and "Control DNA 9947A" among the components of the kit. A recommended range of "input sample DNA" is set forth. Clearly these are reagents for performing a PCR amplification reaction. While the control DNA is provided, the test DNA is not in the kit and is provided by the particular lab where the kit will be used. The primers will anneal to this test DNA as part of the PCR reaction. The kits are indicated as able to "amplify and type" in the context of an ABI PRISM machine. *AmpFlSTR® Profiler® PCR Amplification Kit, Product P/N 403038 Insert P/N 4304212 REV J*.

103. The Product Insert for the AB Profiler® PCR Amplification Kit (Exhibit 62) indicates that the kit includes dye-labeled primers for nine STR loci (plus a gender marker) in a single tube: "One tube of locus-specific 5-FAM-, JOE-, and NED-labeled and unlabeled primers in buffer to amplify the STR loci D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, and D7S820, and the gender marker amelogenin. *AmpFlSTR® Profiler® PCR Amplification Kit, Product P/N 403038 Insert P/N 4304212*

REV J. All of the STRs amplified by this kit contain tetranucleotide repeats. *AmpFlSTR® Profiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems, pg 9-16*.

104. The User's Manual for the AB Profiler® PCR Amplification Kit (Exhibit 63) indicates that the dyes are fluorescent dyes: "PCR-amplified STR alleles can be detected using various methods, such as fluorescent dye labeling …" *AmpFlSTR® Profiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems, pg 1-1*. (underlining added).

105. The User's Manual for the AB Profiler® PCR Amplification Kit (Exhibit 63) indicates that the kit employs allelic ladders to type the samples: "The AmpFlSTR® Allelic Ladders are used to genotype the analyzed samples. The alleles contained in the allelic ladders and the genotype of the AmpFlSTR® Control DNA 9947A are listed in Table 1-3." *AmpFlSTR® Profiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 1-8 [underlining added].

106. The User's Manual for the Profiler® PCR Amplification Kit (Exhibit 63) provides instructions for using the components of the kit to perform a multiplex polymerase chain reaction: "… protocols for PCR amplification of the AmpFlSTR Profiler loci." *AmpFlSTR® Profiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 1-4 [underlining added]

107. The User's Manual for the Profiler® PCR Amplification Kit (Exhibit 63) provides instructions for using the components of the kit to "co-amplify" STR loci: "The AmpFlSTR Profiler PCR Amplification Kit co-amplifies the repeat regions of the following nine short tandem repeat loci …" *AmpFlSTR® Profiler® PCR Amplification*

Kit User's Manual Copyright 2006, 2010 Applied Biosystems, pg 1-5 [underlining added].

ABI COfiler® Kit

108. The Product Insert for the AB COfiler® Kit (Exhibit 64) indicates the kit contains "PCR Reaction Mix," dye-labeled primers, DNA polymerase, and "Control DNA 9947A." Clearly these are reagents for performing a PCR amplification reaction. A recommended range of "input sample DNA" is set forth. While the control DNA is provided, the test DNA is not in the kit and is provided by the particular lab where the kit will be used. The primers will anneal to this test DNA as part of the PCR reaction. The kits are indicated as able to "amplify and type" in the context of an ABI PRISM machine. *AmpFlSTR® COfiler® PCR Amplification Kit, Product P/N 4305246 Insert P/N 4305253 REV K.*

109. The User's Manual for the AB COfiler® Kit (Exhibit 65) indicates the kit "co-amplifies the repeat regions of the following six tetranucleotide short tandem repeat loci: D3S1358, D16S539, TH01, TPOX, CSF1PO, and D7S820." *AmpFlSTR® COfiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 1-5.

110. The User's Manual for the AB COfiler® Kit (Exhibit 17) indicates the kit is to be used "in conjunction with the AmpFlSTR® Profiler Plus™ PCR Amplification Kit to amplify the selected 13 STR loci in two PCR reactions." *AmpFlSTR® COfiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 1-1 (underlining added).

111. The User's Manual for the AB COfiler® Kit (Exhibit 17) indicates an allelic ladder is provided in the kit and that it "contains the most common alleles for each locus." *AmpFlSTR® COfiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 2-3.

AB Profiler Plus® Kit

112. The User's Manual for the AB Profiler Plus® PCR Amplification Kit (Exhibit 66) lists "PCR Reaction Mix" (which contains the triphosphates in a PCR buffer), primer sets, AmpliTaq Gold® DNA Polymerase, and "Control DNA 9947A" among the components of the kit. (Table 1-2) A recommended range of "input sample DNA" is set forth. (p.1-9) Clearly these are reagents for performing a PCR amplification reaction. While the control DNA is provided, the test DNA is not in the kit and is provided by the particular lab where the kit will be used. The primers will anneal to this test DNA as part of the PCR reaction. The kits are indicated as able to "amplify and type" the recommended amount of sample DNA. (p. 12-10) *AmpFlSTR® Profiler Plus® PCR Amplification Kit User's Manual, Product* P/N 43033.

113. The User's Manual for the AB Profiler® PCR Amplification Kit (Exhibit 66) indicates that the kit includes dye-labeled primers for nine STR loci (plus a gender marker) in a single tube: "One tube of locus-specific 5-FAM-, JOE-, and NED-labeled and unlabeled primers in buffer to amplify the STR loci D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820, and the gender marker amelogenin." (Table 1-2) All of the STRs amplified by this kit contain tetranucleotide repeats. (Table 1-1)

114. The User's Manual for the AB Profiler Plus® PCR Amplification Kit (Exhibit 66) indicates that the dyes are fluorescent dyes: "The fluorescent dyes attached to the primers are light-sensitive." (p. 5-2)

115. The User's Manual for the AB Profiler Plus® PCR Amplification Kit (Exhibit 66) indicates that the kit employs three allelic ladders to type the samples: "The AmpFlSTR Allelic Ladders are used to genotype the analyzed samples. The alleles contained in the allelic ladders and the genotype of the AmpFlSTR Control DNA 9947A are listed in Table 1-3." [underlining added]

116. The User's Manual for the Profiler Plus® PCR Amplification Kit (Exhibit 66) provides instructions for using the components of the kit to perform a multiplex polymerase chain reaction in the protocol starting on page 5-2.

117. The User's Manual for the Profiler Plus® PCR Amplification Kit (Exhibit 66) provides instructions for using the components of the kit to "co-amplify" STR loci: "The AmpFlSTR Profiler Plus PCR Amplification Kit contains the PCR reagents necessary to co-amplify the ten AmpFlSTR Profiler Plus loci." (p. 1-7)

AB Yfiler® Kit

118. The Product Insert for the AB Yfiler® Kit (Exhibit 67) lists "PCR Reaction Mix" (with dNTPs in a PCR buffer), dye-labeled primer sets, AmpliTaq Gold DNA Polymerase, and "Control DNA 007." Clearly these are reagents for performing a PCR amplification reaction. While the control DNA is provided, the test DNA is not in the kit and is provided by the particular lab where the kit will be used. The primers will anneal to this test DNA as part of the PCR reaction. The kits are indicated as able to

"amplify and type" in the context of an ABI PRISM machine. *AmpFlSTR® Yfiler® PCR Amplification Kit, Product P/N 4359513 Insert P/N 4359563 REV G*.

119. The Product Insert for the Yfiler® PCR Amplification Kit (Exhibit 67) indicates that the kit amplifies 15 STR loci found on the human Y chromosome. *AmpFlSTR® Yfiler® PCR Amplification Kit, Product P/N 4359513 Insert P/N 4359563 REV G*

120. The User's Manual for the Yfiler® PCR Amplification Kit (Exhibit 68) indicates that loci have repeat sequences of 3 to 6 bases that are tandemly repeated. For example, it indicates that the DYS385 14.2 allele contains 14 complete four base pair repeat units and a partial repeat unit of two base pairs. *AmpFlSTR® Yfiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, pg 5-53.

121. The User's Manual for the Yfiler® PCR Amplification Kit (Exhibit 68) indicates the alleles, and these include both simple and cryptically simple DNA sequences. For example, DYS458 contains simple DNA sequence repeats of $[GAAA]_{13}$ to $[GAAA]_{20}$ (http://www.cstl.nist.gov/strbase/str_y458.) *AmpFlSTR® Yfiler® PCR Amplification Kit User's Manual Copyright 2006, 2010 Applied Biosystems*, p. 6-26.

F. The Accused Products Have the Technical Elements Specified in the Asserted Kit Claims

122. I understand that Promega is asserting that a number of claims of the RE37,984 Patent are infringed by Life Tech/AB when certain products are sold outside the licensed fields. Claim 42 is a kit claim which specifies five (5) elements:

a vessel comprising a mixture of primers,

a vessel containing a polymerase,

a vessel containing triphosphates,

a vessel containing a buffer for performing PCR and

a vessel containing control template DNA comprising i) simple or cryptically simple repeats, the repeat motif of 3 to 10 nucleotides in length and ii) flanking sequences for annealing at least one pair of primers.

All of the accused products provide these reagents. The Insert for the Identifiler® Kit lists "PCR Reaction Mix" (which contains the triphosphates in a buffer solution to support PCR satisfying components c) and d)), along with a) primer sets to amplify STR loci, b) AmpliTaq Gold® DNA Polymerase, and e) control DNA. As noted above, the COfiler® Kit, Profiler Plus® and Yfiler® Kit contain these components. The Profiler® Kit also contains these reagents. To the extent the triphosphates and buffer in these kits are not in separate vessels, there is no significant technical difference, since they will all eventually be mixed together in the PCR reaction.

123. Part e) of Claim 42 of the '984 Patent indicates that the template DNA in the kit must be such that repeats of 3 to 10 nucleotides in length can be amplified with the primers in the kit (although no specific loci are mentioned in Claim 42). As noted above, the accused AB/LT kits contain control DNA template which the primers can amplify. More specifically, as noted above, the User's Manual for the Identifiler® Kit (Exhibit 61) indicates that the control DNA can be amplified with the primers to generate "alleles."

124. Part e) of Claim 42 specifies repeats and repeat "motifs" (a term I explained above, at paragraph 77). All of the accused products amplify a locus which has either simple or cryptically simple sequence repeats. For example, the COfiler® Kit includes the TPOX locus among the loci amplified. The TPOX locus has many alleles which are simple $(AATG)_n$ repeats. In addition, the Identifiler® Kit and the Profiler Plus® Kit include the D21S11 locus among the loci amplified (as noted previously, all of the alleles at this locus are cryptically simple repeats of various types). The Identifier® Kit, the Profiler® Kit, and the Profiler Plus® Kit include the D5S818 locus among the loci amplified (as noted previously, all of the alleles with sequences shown are simple repeats). The alleles amplified by the Yfiler® PCR Amplification Kit have repeat sequences of 3 to 6 bases that are tandemly repeated; the alleles include both simple and cryptically simple DNA sequences. All of the STRs amplified by the kits described above consist of simple or cryptically simple DNA repeats of 3-6 nucleotides. Therefore, I conclude that the accused products provide the technical elements set forth in Claim 42 of the '984 Patent.

Claim 5 of the '771 Patent

125. I understand that the only asserted Claim of U.S. Patent No. 7,008,771 is Claim 5 and that Promega asserts this claim is directly infringed by the Life Tech/AB Identifiler® product (when sold outside the licensed fields). Claim 5 is a kit claim which recites in part: "A kit for simultaneously analyzing a set of loci of genomic DNA, comprising oligonucleotide primers for co-amplifying a set of loci of the Genomic DNA to be analyzed, wherein the primers are in one or more containers, wherein the primers

are designed to co-amplify a set of loci from one or more DNA samples, comprising short tandem repeat loci . . . [list of 13 particular loci] and a locus selected from the group consisting of G475 . . .and Amelogenin." (Claim 5, underlining added).

126. I have looked at the technical elements specified in Claim 5. Claim 5 specifies 13 particular loci (along with an additional locus which can be Amelogenin) to be co-amplified. The Life Tech/AB Identifiler® product is indicated to be a "Fluorescent STR kit" that includes, among other things, primers for co-amplifying "15 STR loci" which include the 13 recited STR loci in Claim 5 and the Amelogenin locus. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 5 of the '771 Patent.

127. So that there is no confusion, I note that, while Claim 5 specifies "HUMCSF1PO," HUMTH01," and "HUMTPOX," these loci are also known simply as CSF1PO, TH01 and TPOX. While Claim 5 specifies "HUMFIBRA," this locus is also known simply as FGA. While Claim 5 specifies HUMvWFA31," this locus is also known simply as vWA. The AB kits use the shorter name for these loci.

Claims 18-19 and 21-23 of the '235 Patent

128. I understand that Promega asserts that Claims 1, 4, 6-13, 15-19, and 21-23 of U.S. Patent No. 6,479,235 are directly infringed by the Life Tech/AB Identifiler® product when sold outside the licensed fields. I have looked at the technical elements specified in these claims. Claim 18 is a kit claim which recites in part: "A kit for simultaneously analyzing a set of loci of genomic DNA comprising oligonucleotide primers for co-amplifying a set of loci of the genomic DNA to be analyzed, wherein the

set of loci comprises short tandem repeat loci which can be co-amplified, the primers are in one or more containers, the genomic DNA is human genomic DNA, and the loci comprise . . . . [list of 13 particular loci]. (Claim 18, underlining added). Claim 18 specifies 13 particular loci to be co-amplified. The Life Tech/AB Identifiler® product is indicated to be a "Fluorescent STR kit" that includes, among other things, primers for co-amplifying "15 STR loci" which include the 13 required STR loci in Claim 18 plus amelogenin. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 18 of the '235 Patent.

129. So that there is no confusion, I again note that, while Claim 18 specifies "HUMCSF1PO," HUMTH01," and "HUMTPOX," these loci are also known simply as CSF1PO, TH01 and TPOX. While Claim 18 specifies "HUMFIBRA," this locus is also known simply as FGA. While Claim 18 specifies HUMvWFA31," this locus is also known simply as vWA. The AB kit refers to the short name for these loci.

130. Claim 19 of the '235 Patent depends on Claim 18 and adds the feature that all of the primers are in one container. As noted previously, the primers in the Identifiler® product kit are in "one tube". Therefore, I conclude that the accused product provides the technical elements set forth in Claim 19 of the '235 Patent.

131. Claim 21 of the '235 Patent depends on Claim 18 and adds the feature of "reagents for at least one multiplex amplification reaction." Such reagents would include a polymerase and triphosphates. As noted above, the Identifiler® Kit contains such reagents. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 21 of the '235 Patent.

132. Claim 22 of the '235 Patent depends on Claim 18 and adds the feature of an "allelic ladder." The Identifiler® Kit also specifies an "Allelic Ladder". Therefore, I conclude that the accused product provides the technical elements set forth in Claim 22 of the '235 Patent.

133. Claim 23 of the '235 Patent depends on Claim 22 and adds the feature that the rungs of the allelic ladder and at least one primer have a fluorescent label covalently attached, and at least two primers have different labels. The Identifier® Kit provides allelic ladder rungs and primers that are covalently labeled with fluorescent dyes, including primers with different labels. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 23 of the '235 Patent.

Claim 25 and 27-31 of the '660 Patent

134. I understand that Promega asserts that Claims 2-5, 9, 16-17, 19-21, 23-25, and 27-31 of U.S. Patent No. 5,843,660 are directly infringed by the Life Tech/AB Identifiler® product when sold outside the licensed fields. I have looked at the technical elements specified in these claims. Claim 25 is a kit claim which recites in part: "A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising a container which has oligonucleotide primers for co-amplifying a set of at least three short tandem repeat loci, wherein the set of loci are selected from the sets of loci consisting of: [list of 29 sets of loci]. (Claim 25, underlining added). While 29 sets of loci are set forth in Claim 25, one of the 29 sets specifies that the three loci are D16S539, D7S820, and D13S317. As noted above, the Identifiler® Kit contains primers in a single tube to co-amplify loci combinations including D7S820, D13S317, and

D16S539. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 25 of the '660 Patent.

135. Claim 27 depends on Claim 25 and further specifies "a container having reagents for at least one multiplex amplification reaction." As noted above, the Identifiler® Kit provides multiplex amplification reaction reagents. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 27 of the '660 Patent.

136. Claim 28 depends on Claim 25 and further specifies "a container having an allelic ladder." As noted previously, the Identifiler® Kit provides a container having an allelic ladder. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 28 of the '660 Patent.

137. Claim 29 depends on Claim 28 and further specifies "each rung of the allelic ladder and at least one primer … have a label covalently attached." As noted previously, the Identifiler® Kit provides allelic ladder rungs and primers that are covalently labeled. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 29 of the '660 Patent.

138. Claim 30 depends on Claim 29 and further specifies "the label is a fluorescent label." As noted previously, the Identifiler® Kit provides reagents with fluorescent labels. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 30 of the '660 Patent.

139. Claim 31 depends on Claim 30 and further specifies "at least one of the … primers … has a different fluorescent label …". As noted previously, the Identifier® Kit provides at least one primer fluorescent label that is different from another primer

fluorescent label. Therefore, I conclude that the accused product provides the technical elements set forth in Claim 31 of the '660 Patent.

Claims 10, 23-24, 27 and 33 of the '598 Patent

140. I understand that Promega asserts that Claims 1-2, 4-10, 12, 15, 19, 21-24, 27-28, and 31-33 of U.S. Patent No. 6,221,598 are directly infringed by some ABI products, including the Life Tech/ABI Identifiler® product and Profiler® Kit when sold outside the licensed fields. I have looked at the technical elements specified in these claims. Claim 10 is a kit claim which recites in part: "A kit for simultaneously analyzing short tandem repeat sequences in at least three loci, comprising: a single container containing oligonucleotide primers for each locus in a set of at least three short tandem repeat loci, wherein the at least three short tandem repeat loci in the set comprises at least three loci selected from the group consisting of: [list of 20 sets of loci]. (Claim 10, underlining added). While 20 different sets of loci are listed in Claim 10, one set specifies that the loci include at least HUMTPOX, HUMVWFA31 and HUMCSF1PO (in a first case), and (in another case) HUMCSF1PO, HUMTH01 and HUMVWFA31 (among a number of possible sets). As noted previously, simpler names for these loci are TPOX, vWA and CSF1PO (in the one case) and CSF1PO, TH01 and vWA (in the other). The Identifiler® Kit and the Profiler® Kit provide a single container with primers for these two combinations of three loci (as well as additional loci). Therefore, I conclude that the Identifiler® Kit and the Profiler® Kit provide the technical elements set forth in Claim 10 of the '598 Patent.

141. Claim 23 of the '598 Patent is also a kit claim which specifies in part: "A kit for simultaneously analyzing short tandem repeat sequences in a set of short tandem repeat loci from one or more DNA samples, comprising: a single container containing oligonucleotide primers for each locus in a set of short tandem repeat loci which can be co-amplified, comprising, HUMCSF1PO, HUMTPOX and HUMTH01." (Claim 23, underlining added). Claim 23 indicates the loci include HUMCSF1PO, HUMTPOX, and HUMTH01 (or more simply, CSF1PO, TPOX and TH01). The COfiler® Kit co-amplifies TPOX, TH01 and CSF1PO (along with other loci) which are the loci specified in Claim 23 (see paragraph 119). The Identifiler® Kit and the Profiler® Kit provide a single container with primers for these three loci (as well as additional loci). Therefore, I conclude that the Identifiler® Kit, COfiler® Kit and the Profiler® Kit provide the technical elements set forth in Claim 23 of the '598 Patent.

142. Claim 24 depends on Claim 23 and further specifies that the kit contains primers designed to co-amplify the HUMVWFA31 (or more simply vWA) locus. The Identifiler® Kit and the Profiler® Kit are identified as providing a single container with primers for this locus as well. Therefore, I conclude that the Identifiler® Kit and the Profiler® Kit provide the technical elements set forth in Claim 24 of the '598 Patent.

143. Claim 27 depends on Claim 23 and specifies "at least one of each of the pair of oligonucleotide primers in the kit is fluorescently-labeled." As noted previously, the Identifiler® Kit, COfiler® Kit and the Profiler® Kit are identified as providing fluorescently labeled primers. Therefore, I conclude that the Identifiler® Kit, COfiler® Kit and the Profiler® Kit provide the technical elements set forth in Claim 27 of the '598 Patent.

144. Claim 33 is another independent kit claim of the '598 Patent and it recites in part: "A kit for simultaneously analyzing short tandem repeat sequences in a set of short tandem repeat loci from one or more DNA samples, comprising: a single container containing oligonucleotide primers for each locus in a set of short tandem repeat loci which can be co-amplified, comprising, HUMTPOX, HUMVWFA31, and HUMCSF1PO." (Claim 33, underlining added). Claim 33 indicates that the loci include HUMCSF1PO, HUMTPOX and HUMVWFA31 (or more simply, CSF1PO, TPOX and vWA). The Identifiler® Kit and the Profiler® Kit are identified as providing a single primer container for these loci. Therefore, I conclude that the Identifiler® Kit and the Profiler® Kit provide the technical elements set forth in Claim 33 of the '598 Patent.

G. The Accused Products Provide the Technical Elements of the Method Claims

Claims 15-16, 18, 23, 25, 27-28 and 41 of the '984 Patent

145. I understand that Promega is asserting that a number of method claims of the RE37,984 Patent are infringed by Life Tech/AB when certain products are sold outside the licensed fields. Claim 15 is a method claim which recites in part: "A method for analyzing length polymorphisms in at least one locus in an DNA sample . . . wherein said DNA sample comprises a DNA template having at least one locus comprising a simple or cryptically simple DNA sequence, said method comprising: a) annealing said DNA template with at least one pair of primers, wherein said primer pair is composed of a first primer complementary to a nucleotide sequence flanking said simple or cryptically simple DNA sequence on the 5' side of said simple or cryptically simple DNA sequence

and a second primer complementary to a nucleotide sequence flanking the simple or cryptically simple DNA sequence on the 3' side of said simple or cryptically simple DNA sequence; wherein said first and second primers each anneal to a single site in said DNA template and wherein the annealing sites are separated by 50 to 500 nucleotides of template DNA; b) performing at least one primer-directed polymerase chain reaction upon said template DNA having said primers annealed thereto, so as to form at least one polymerase chain reaction product; c) separating the products of each polymerase chain reaction according to their lengths; and d) analyzing the lengths of the separated products to determine the length polymorphisms of said simple or cryptically simple sequences; wherein said simple or cryptically simple sequence has a repeat length of 3 to 10 nucleotides."

146. I have discussed the meaning of "simple" and "cryptically simple sequences" shown underlined above. As noted above in the context of Claim 42 of the '984 Patent, all of the accused products amplify a locus which has either simple or cryptically simple sequence repeats. For example, the COfiler® Kit includes the TPOX locus among the loci amplified. The TPOX locus has many alleles which are simple $(AATG)_n$ repeats. In addition, the Identifiler® Kit and the Profiler Plus® Kit include the D21S11 locus among the loci amplified (as noted previously, all of the alleles at this locus are cryptically simple repeats of various types). The Identifier® Kit, the Profiler® Kit, and the Profiler Plus® Kit include the D5S818 locus among the loci amplified (as noted previously, all of the alleles with sequences shown are simple repeats). The alleles amplified by the Yfiler® PCR Amplification Kit have repeat sequences of 3 to 6 bases

that are tandemly repeated; the alleles include both simple and cryptically simple DNA sequences.

147. I have also discussed the steps of a) annealing and b) performing a PCR reaction, (see underlining in Claim 15 above) in general terms previously (see paragraphs 78-79). These steps are specified for users of the accused kits. For example, the COfiler® Kit is a PCR amplification kit with primers for annealing, as part of the PCR reaction. Similarly, the Identifiler® Kit, Profiler® Kit, and the Profiler Plus® Kit provide reagents for PCR, including primers for annealing to template as part of the PCR reaction. Finally, the Yfiler® Kit is a PCR amplification kit with primers for annealing, as part of the PCR reaction . Figure 3-6 of the Identifiler Users Manual shows that the allele sizes of the amplified STRs range from about 107 to 340 nucleotides in length meaning that the primer annealing sites are separated by slightly less than that distance. Similarly Figure 5-3 of the Yfiler User's Manual (Exhibit 68) demonstrates that the allele sizes range from about 100 to 330 nucleotides in length.

148. I have also discussed the steps of c) separating the PCR products, and d) analyzing the separated products to determine length polymorphisms which are underlined above in Claim 15. These steps are specified for users of the accused kits. For example, the COfiler® Kit is a PCR amplification kit which is indicated as able to "amplify and type" in the context of an ABI PRISM machine. The Yfiler® Kit is a PCR amplification kit which is indicated as able to "amplify and type" in the context of an ABI PRISM machine. Similarly, the Identifiler® Kit, Profiler® Kit and the Profiler Plus® Kit are indicated as able to "amplify and type" in the context of an ABI PRISM machine. The User Guide for the Identifiler® Kit indicates that the PCR products are

"electrophoretically separated" and "detected" on the ABI PRISM instrument. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 15 of the '984 Patent.

149. Claim 16 of the '984 Patent depends on Claim 15 and further specifies that the repeat length is between 3 and 6 nucleotides. I discussed repeat lengths of the loci amplified by the kits in the context of the length range of 3 to 10 nucleotides for Claim 15. Claim 16 simply is a narrower range. Nonetheless, the kits amplify loci with repeat lengths in this range. For example, as noted previously, the alleles amplified by the Yfiler® PCR Amplification Kit have repeat sequences of 3 to 6 bases that are tandemly repeated; the alleles include both simple and cryptically simple DNA sequences. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 16 of the '984 Patent.

150. Claim 18 of the '984 Patent depends on Claim 15 and further specifies that "at least two primer pairs are used." As noted previously, the accused kits have many primer pairs for amplification of many loci. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 18 of the '984 Patent.

151. Claim 23 of the '984 Patent depends on Claim 15 and further specifies that "2 to 50 primer pairs are used." As noted previously, the accused kits have primer pairs for amplification of loci, the number of which falls within this range. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler®

Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 23 of the '984 Patent.

152. Claim 25 depends on Claim 15 and further specifies that "each of the products of the primer-directed polymerase chain reaction is separable one from the other as individual bands on a suitable electrophoretic gel." As noted above, each of the accused products is able to amplify and type in the context of an ABI PRISM instrument and this instrument is used to "electrophoretically separate" and "detect" the amplified products using a "five-dye fluorescent system". Thus, size and color signal are used together to separate "one from the other." Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 25 of the '984 Patent.

153. Claim 27 depends on Claim 15 and specifies that the PCR product "is labeled by a non-radioactive label." As noted above, each of the accused products utilizes dye-labeled primers, which will generate a PCR product labeled with a non-radioactive label. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 27 of the '984 Patent.

154. Claim 28 depends on Claim 27 and specifies that the "non-radioactive label is a fluorescent label." As noted above, each of the accused products utilizes dye-labeled primers. Moreover, these dyes are fluorescent. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 28 of the '984 Patent

155. Claim 41 of the '984 Patent is a method claim which recites in part: "A method for analyzing polymorphism in at least one locus in an DNA sample comprising a DNA template, said method comprising: a) annealing said DNA template with at least one pair of primers, wherein said primer pair is composed of a first primer complementary to a nucleotide sequence flanking said simple or cryptically simple DNA sequence on the 5' side of said simple or cryptically simple DNA sequence and a second primer complementary to a nucleotide sequence flanking the simple or cryptically simple DNA sequence on the 3' side of said simple or cryptically simple DNA sequence; wherein said first and second primers each anneal to a single site in said DNA template and wherein the annealing sites are separated by 50 to 500 nucleotides of template DNA; b) performing at least one primer-directed polymerase chain reaction upon said template DNA having said primers annealed thereto, so as to form at least one polymerase chain reaction product; c) separating the products of each polymerase chain reaction product according to their lengths; and d) analyzing the lengths of the separated products to determine the length polymorphisms of said simple or cryptically simple sequences, wherein said DNA template includes at least one sequence consisting essentially of a simple or cryptically simple DNA sequence having a repeat motif length of 3 to 10 nucleotides and nucleotide sequences flanking said simple or cryptically simple DNA sequence effective for annealing said at least one pair of primers."

156. I have discussed the meaning of "simple" and "cryptically simple sequences" shown underlined above (see paragraphs 83-84). As noted above in the context of Claim 42 of the '984 Patent, all of the accused products amplify a locus which has either simple or cryptically simple sequence repeats. For example, the COfiler® Kit

includes the TPOX locus among the loci amplified. The TPOX locus has many alleles which are simple $(AATG)_n$ repeats. In addition, the Identifiler® Kit and the Profiler Plus® Kit include the D21S11 locus among the loci amplified (as noted previously, all of the alleles at this locus are cryptically simple repeats of various types). The Identifier® Kit, the Profiler® Kit, and the Profiler Plus® Kit include the D5S818 locus among the loci amplified (as noted previously, all of the alleles with sequences shown are simple repeats). The alleles amplified by the Yfiler® PCR Amplification Kit have repeat sequences of 3 to 6 bases that are tandemly repeated; the alleles include both simple and cryptically simple DNA sequences. All of the other kits analyze STRs with tetranucleotide repeats.

157. I have also discussed the steps of a) annealing and b) performing a PCR reaction, (see underlining in Claim 41 above) in general terms previously. These steps are specified for users of the accused kits. For example, the COfiler® Kit is a PCR amplification kit with primers for annealing, as part of the PCR reaction. Similarly, the Identifiler® Kit, Profiler® Kit and the Profiler Plus® Kit provide reagents for PCR, including primers for annealing to template as part of the PCR reaction. Finally, the Yfiler® Kit is a PCR amplification kit with primers for annealing, as part of the PCR reaction.

158. I have also discussed the steps of c) separating the PCR products, and d) analyzing the separated products to determine length polymorphisms which are underlined above in Claim 41. These steps are specified for users of the accused kits. For example, the COfiler® Kit is a PCR amplification kit which is indicated as able to "amplify and type" in the context of an ABI PRISM machine. The Yfiler® Kit is a PCR

amplification kit which is indicated as able to "amplify and type" in the context of an ABI PRISM machine. Similarly, the Identifiler® Kit, Profiler® Kit and the Profiler Plus® Kit are indicated as able to "amplify and type" in the context of an ABI PRISM machine. The User Guide for the Identifiler® Kit indicates that the PCR products are "electrophoretically separated" and "detected" on the ABI PRISM instrument. Therefore, I conclude that the Identifiler® Kit, the Profiler® Kit, the Profiler Plus® Kit, the Yfiler® Kit, and the COfiler® Kit all provide the technical elements set forth in Claim 41 of the '984 Patent.

159. Claims 1, 12, and 28 are independent method claims of the '598 Patent. Claim 1 recites in part a method having the following steps:

"a)obtaining at least one DNA sample to be analyzed; b) selecting a set of at least three short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, wherein the at least three short tandem repeat loci in the set comprises at least three loci selected from the group consisting of: [list of 18 sets of loci]; c) co-amplifying . . . thereby producing a mixture of amplified alleles . . .; and d) evaluating the amplified alleles . . . to determine the alleles present at each of the co-amplified loci in the set."

160. Claim 12 is similar to Claim 1, but differs in that it only sets forth a single set of loci. Claim 12 recites in part:

"a) obtaining at least one DNA sample to be analyzed; b) selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, comprising HUMCSF1PO, HUMTPOX, and HUMTH01; c) co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and evaluating the

amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

161. Claim 28 is similar to Claim 12 in that it also sets forth only a single set of loci. Claim 28 recites in part:

"a) obtaining at least one DNA sample to be analyzed; b) selecting a set of short tandem repeat loci of the DNA sample to be analyzed which can be co-amplified, comprising HUMTPOX, HUMVWFA31, and HUMCSF1PO; c) co-amplifying the set of short tandem repeat loci in a multiplex amplification reaction, thereby producing a mixture of amplified alleles from each of the co-amplified loci in the set; and d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the co-amplified loci in the set.

162. From the above, it is clear that Claims 1, 12 and 28 have some common steps with some language in common (which is underlined above). With regard to step a), Claims 1, 12, 28 specify "obtaining at least one DNA sample." All products accused of infringing the '598 Patent utilize at least one DNA sample in their protocols. Indeed, the Identifiler® Kit, the Profiler® Kit, and the COfiler® Kit all provide a recommended range of "input sample DNA.". Therefore, I conclude that the technical elements for step a) of these claims are found in the accused kits.

163. With regard to step b), Claims 1, 12 and 28 specify "selecting a set of . . . short tandem repeat loci … which can be co-amplified." Specifically, claim 1 specifies in step b) that the "at least three loci selected" are from a group which includes in one case HUMTPOX, HUMTH01, and HUMVWFA31, and in another case HUMCSF1PO, HUMTH01, and HUMVWFA31 (among a number of possible sets of loci listed). Claim

12 specifies in step b) that the set of loci selected comprises HUMCSF1PO, HUMTPOX, and HUMTH01. Claim 28 specifies in step b) that the set of loci selected comprises HUMTPOX, HUMVWFA31, and HUMCSF1PO. All products accused of infringing the '598 Patent utilize at least three short tandem repeat loci which can be co-amplified in their protocols. The COfiler® Kit co-amplifies TPOX, TH01 and CSF1PO (along with other loci), which is the precise combination specified in Claim 12. The Identifiler® Kit and the Profiler® Kit are identified as co-amplifying short tandem repeat loci which include at least one of the sets of loci found in each of claims 1, 12, and 28. Therefore, I conclude that the technical elements for step b) of these claims are found in the accused kits.

164. With regard to step c), Claims 1, 12 and 28 further specify "co-amplifying . . . , thereby producing a mixture of amplified alleles . . " I have noted that the Identifiler® Kit, the COfiler® Kit, and the Profiler® Kit all "co-amplify" to produce a mixture of alleles. Therefore, I conclude that the technical elements for step c) of these claims are found in the accused kits.

165. With regard to step d), Claims 1, 12 and 28 further specify "evaluating the amplified alleles . . . to determine the alleles present . . ." I have previously indicated that the Identifiler® Kit, the COfiler® Kit, and the Profiler® Kit are able to "amplify and type" in the context of an ABI PRISM machine and thereby accurately characterize the alleles present. Therefore, I conclude that the technical elements for step d) of these claims are found in the accused kits.

166. Claim 2 of the '598 depends on Claim 1 and specifies that the loci "are co-amplified by multiplex polymerase chain reaction." I have discussed the difference

between "monoplex" and "multiplex" (paragraph 80 above) PCR reactions. The User Manual for the ABI Identifiler® Kit (Exhibit 61) makes this distinction. I have previously indicated that the Identifiler® Kit, the COfiler® Kit, and the Profiler® Kit provide reagents for performing a multiplex PCR amplification reaction. Therefore, I conclude that the technical elements for Claim 2 of the '598 are found in the accused kits.

167. Claim 4 of the '598 depends on Claim 1 and specifies that the alleles are evaluated by comparing the alleles to a size standard or a locus-specific allelic ladder. I have previously indicated that the Identifiler® Kit, the COfiler® Kit, and the Profiler® Kit each provide an allelic ladder including the most common alleles for each locus (i.e. it is locus specific). The kits also list a size standard as "required materials." Page 3-22 of the Identifiler User Manual provides the protocol for preparing and analyzing amplified PCR products using both the size standard and the allelic ladder. The other kits are utilized in a similar fashion. Therefore, I conclude that the technical elements for Claim 4 of the '598 are found in the accused kits.

168. Claims 15 and 21 of the '598 specify that the set of loci co-amplified further comprises HUMVWFA31. I have noted previously that this locus is also known simply as vWA. The AB kits refer to the short name for this locus. The Identifiler® Kit and the Profiler® Kit also co-amplify this locus. Therefore, I conclude that the technical elements for Claims 15 and 21 of the '598 are found in the accused kits.

169. Claim 19 depends on Claim 12 and specifies "oligonucleotide primers for each locus in the set of loci selected in step (b), wherein at least one of the oligonucleotide primers for each locus is fluorescently labeled." The Identifiler® Kit, COfiler® Kit and the Profiler® Kit provide oligonucleotide primers for each locus. The

Identifiler® Kit, COfiler® Kit and the Profiler® Kit indicate the primers are fluorescently labeled. Therefore, I conclude that the technical elements for Claim 19 of the '598 are found in the accused kits.

Claims of the 660 Patent

170. Claim 16 of the '660 is a method claim which reads in part: "comprising: obtaining at least one DNA sample to be analyzed, selecting a set of three short tandem repeat loci of the DNA sample to be analyzed which can be amplified together, wherein the set of three loci is selected from the group of sets of loci consisting of [six sets of loci]; co-amplifying the three loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample." Claim 1 (not asserted) of the '660 is similar except for the fact that it specifies "a set of at least four short tandem repeat loci."

171. Claims 2, 3, 4 and 5, each of which depends on Claim 1 (not asserted), and claim 16 each specify at step (a) "obtaining at least one DNA sample . . .". I have previously noted that the Identifiler® kit utilizes at least one DNA sample in its protocols. Indeed, the Identifiler® Kit provides a recommended range of "input sample DNA." (see paragraphs 97, above). Therefore, I conclude that the technical elements for step a) of these claims are found in the accused kit.

172. Claims 2, 3, 4 and 5 and 16 specify the following at step b):

Claim 2: selecting "a set of at least four loci" from a "group of sets of loci" that include in one case "D7S820, D13S317, D16S539, HUMvWFA31."

Claim 3: selecting "a set of at least six loci" from a "group of sets of loci" that include in, one case, "D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, and HUMTPOX."

Claim 4: selecting "a set of at least seven loci" from a "group of sets of loci" that include in, one case, "D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, and HUMTH01."

Claim 5: selecting "a set of at least eight loci" from a "group of sets of loci" that include in, one case, "D16S539, D7S820, D13S317, D5S818, HUMCSF1PO, HUMTPOX, HUMTH01 and HUMvWFA31."

Claim 16: selecting "a set of at least three loci" from a "group of sets of loci" that include in, one case, "D16S539, D7S820, D13S317."

173. The Identifiler® Kit co-amplifies loci that include each of the above identified sets of loci regarding claims 2-5 and 16. Therefore, I conclude that the technical elements for step b) of these claims are found in the accused kit.

174. Claims 2-5 and 16 further specify at step c) "co-amplifying . . ." I have discussed the meaning of amplifying and "co-amplifying". I have also noted that the Identifiler® Kit "co-amplifies" to produce a mixture of alleles. Therefore, I conclude that the technical elements for step c) of these claims are found in the accused kit.

175. Claims 2-5 and 16 further specify at step d) evaluating the amplified alleles . . . to determine the alleles present . . ." I have previously indicated that the Identifiler® Kit is able to "amplify and type" in the context of an ABI PRISM machine and thereby accurately characterize the alleles present. Therefore, I conclude that the technical elements for step d) of these claims are found in the accused kit.

176. Claim 17, which depends on claim 16, specifies that "the amplification is done using three pair of primers, wherein each pair of primers flanks one of the three short tandem repeat loci." I have previously discussed the meaning of flanking regions. The Identifiler® Kit uses more than three primer pairs which flank the repeating

sequence. Otherwise they could not amplify the STR loci they claim as referenced above.

177. Claim 19 depends on Claim 16 and specifies that "the multiplex reaction is a polymerase chain reaction". I have discussed the difference between "monoplex" and multiplex (paragraph 80) PCR reactions. The User Manual for the ABI Identifiler® Kit (Exhibit 61) makes this distinction. I have previously indicated that the Identifiler® Kit provides reagents for performing a multiplex PCR amplification reaction. Therefore, I conclude that the technical elements for Claim 19 of the '660 are found in the accused kit.

178. Claim 20 is dependent from Claim 16 and specifies "comparing separated alleles to a size standard, wherein the size standard is selected from a group of size standards consisting of a DNA marker and a locus-specific allelic ladder. " I have previously indicated that the Identifiler® Kit provides an allelic ladder comprising the most common alleles for each locus (i.e. it is locus specific). The kits also list a size standard as "required materials." The protocol included in the Identifiler® Kit User's Manual uses both to calculate the size of the amplified alleles and identify them. Therefore, I conclude that the technical elements for Claim 20 of the '660 are found in the accused kit.

Claims of the '235 Patent

179. Claims 1 and 13 of the '235 Patent are methods claims. Claim 1 of the '235 patent reads in part:

"a) obtaining at least one DNA sample to be analyzed, b) selecting a set of loci of the

DNA sample, comprising D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31, c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample."

180. Claim 13 of the '235 Patent reads in part:

"a) obtaining at least one DNA sample to be analyzed; b) selecting a set of loci of the DNA sample, comprising short tandem repeat loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31; c) co-amplifying the loci in the set in a multiplex amplification reaction, wherein the product of the reaction is a mixture of amplified alleles from each of the co-amplified loci in the set; and d) evaluating the amplified alleles in the mixture to determine the alleles present at each of the loci analyzed in the set within the DNA sample."

181. With regard to step a) of Claim 1 and 13 of the '235 Patent, I have previously indicated that the Identifiler® Kit provides a recommended range of "input sample DNA." With regard to step b) of Claim 1 and 13 of the '235 Patent, I have previously indicated the Identifiler® Kit provides primers to amplify the loci of step b). With regard to step c) of Claim 1 and 13 of the '235 Patent, I have also noted that the Identifiler® Kit "co-amplifies" to produce a mixture of alleles. With regard to step d) of Claim 1 and 13 of the '235 Patent, I have previously indicated that the Identifiler® Kit is

able to "amplify and type" in the context of an ABI PRISM machine and thereby accurately characterize the alleles present (paragraph 106). Therefore, I conclude that the technical elements for steps a) through d) of these claims are found in the accused kit.

182. Claim 4 depends on Claim 1 of the '235 Patent and specifies that the set of loci "further comprises a locus to identify the gender …". The Identifiler® Kit is identified as providing a gender identifying locus. Therefore, I conclude that the technical elements for Claim 4 are found in the accused kit.

183. Claim 7 depends on Claim 1 and specifies "using pairs of oligonucleotide primers flanking the loci analyzed." As noted previously, the Identifiler® Kit uses primers which flank the repeating sequence. Otherwise they could not amplify the STR loci they claim as referenced above. Therefore, I conclude that the technical elements for Claim 7 are found in the accused kit.

184. Claim 8 depends on Claim 7 and specifies that the set of loci "is co-amplified using a polymerase chain reaction". As noted above, the Identifiler® Kit provides reagents to co-amplify loci using a "polymerase chain reaction". Therefore, I conclude that the technical elements for Claim 8 are found in the accused kit.

185. Claim 9 depends on Claim 7 and specifies that each locus is co-amplified "using a pair of primers which flank the locus wherein at least one primer of each pair has a fluorescent label covalently attached thereto." As noted above, the Identifiler® Kit, uses primers which flank the repeating sequence. Otherwise they could not amplify the STR loci they claim as referenced above. Moreover, I have previously noted that the primers are fluorescently labeled. Therefore, I conclude that the technical elements for Claim 9 are found in the accused kit.

186. Claim 10 depends on Claim 9 and specifies that "at least three" of the labeled primers have different fluorescent labels covalently attached thereto." The Identifiler® Kit is identified as providing that at least three primers are differentially labeled. Therefore, I conclude that the technical elements for Claim 10 are found in the accused kit.

187. Claim 12 depends on Claim 1 and specifies that the amplified alleles are evaluated by comparing to "a size standard, wherein the size standard is selected from a group of size standards consisting of a DNA marker and a locus specific allelic ladder. " I have noted previously that the Identifiler® Kit comes with an allelic ladder whose alleles are locus specific and that a size standard is identified in the "required materials."

188. Claim 15 depends on Claim 13 and specifies that the multiplex amplification reaction "is a polymerase chain reaction." As noted above, the Identifiler® Kit provides reagents to co-amplify loci using a "polymerase chain reaction." Therefore, I conclude that the technical elements for Claim 15 are found in the accused kit.

189. Claim 16 depends on Claim 13 and specifies that the amplified alleles are evaluated by comparing to a "size standard, wherein the size standard is selected from a group of size standards consisting of a DNA marker and a locus specific allelic ladder." As noted above, the Identifiler® Kit comes with a locus specific allelic ladder and that a size standard is identified in the "required materials." Therefore, I conclude that the technical elements for Claim 16 are found in the accused kit.

H. The 2006 License Agreement And The Refusal To Broaden The Fields

190. I was involved in the negotiation of the 2006 License Agreement on behalf of Promega. At one point the parties were having frequent teleconference calls with each other. I raised the question on one of these calls about whether ABI needed or wanted a license for the clinical and research fields. I offered to license ABI in these additional fields. The reply to my offer came in a later teleconference, where Dr. Charles Moehle, who was leading the negotiation on behalf of ABI, indicated ABI had decided not to broaden the fields of use to include these areas.

I. The Promega Patents Are Valid

191. Defendants' expert, Dr. Struhl, has pointed to the following patent and publications (among others):

U.S. Patent No. 5,364,759 ("Caskey")

Chamberlain *et al.*, Deletion Screening of the Duchenne Muscular Dystrophy Locus via Multiplex DNA Amplification, *Nucleic Acids Research* 16:11141-11156 (1988) ("Chamberlain '88")

Chamberlain *et al.*, Multiplex PCR for the Diagnosis of Duchenne Muscular Dystrophy, *in* PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS (1990) "Chamberlain '90")

Kimpton *et al.*, Automated DNA Profiling Employing Multiplex Amplification of Short Tandem Repeat Loci, PCR METHODS AND APPLICATIONS 3:13-22 (1993) ("Kimpton '93")

I will refer to these references as the "Cited Art."

192. The two Chamberlain *et al.* references (see above) do not describe STR multiplex PCR, only standard multiplex PCR. In my opinion, STR multiplex PCR is

much more complex and challenging than standard multiplex PCR. Unlike generic multiplex PCR, STR multiplex PCR must amplify a variety of different length alleles from each locus. Thus, loci not only have to be resolved from one another, but many different alleles from each locus must be resolved in STR multiplex PCR. All of the reaction conditions (discussed more below) must be more carefully optimized so that all of the alleles will be detected and resolved. Additionally, STR alleles at a particular locus only differ by one or a few nucleotides rather than hundreds of nucleotides as in the general mPCR reactions of Chamberlain *et al*. This requires them to be analyzed at high resolution; which creates challenges including the resolution of assay artifacts that do not affect the interpretation of general mPCR. In my opinion, the Promega patents accomplished this result.

a. The‘598 Promega Patent Contains More than the Cited Art and this Material, Which Includes New Multiplexes, Is Not Obvious in View of the Cited Art

193. I have reviewed the Cited Art, including the Caskey patent (see above). The Caskey patent, which appears to have been filed in 1991, describes two multiplexes of two STR loci and one multiplex of three STR loci:

HPRTB and FABP (*see* Caskey Patent, Figure 3A)

RENA4 and TH01 (*see* Caskey Patent, Figure 3B)

TH01, FABP and HPRTB (*see* Caskey Patent, Figure 5)

194. Caskey is a co-author on a 1992 publication by Edwards *et al.* entitled “Genetic Variation at Five Trimeric and Terameric Tandem Repeat Loci in Four Human Population Groups,” *Genomics* 12:241 (1992) which describes the same results for two of

the Caskey Patent multiplexes (each co-amplifying just two STR loci): HPRTB and FABP (*see* Figure 1A); RENA4 and TH01 (*see* Figure 1B). Edwards *et al.* was cited in the '598 Patent.

195. The Kimpton '93 publication describes three multiplexes of STR loci:

vWA31, TH01, F13A1, FES/FPS

CD4, DHFR, CYARO3, APOAII, PLA2A, FIIDA, FABP

GABA, D21S11, ACTBP2

196. Kimpton '93 indicated some problems with at least two of the loci (APOAII and ACTBP2) and acknowledged that "[p]rior to routine use of these loci by forensic laboratories for the identification of individuals, it must be confirmed that the detection and sizing protocols used allow accurate, reliable, and unambiguous allele designation.".

197. The '598 Patent provides thirty-two (32) examples – each with a multiplex: CSFIPO, TPOX, TH01 (Example 1)(Claims 12, 23); CSFIPO, TPOX, TH01, vWA31 (Example 2)(Claims 21, 24, 28, 33); HPRTB, FESFPS, and VWFA31 (Example 3)(Claims 1, 10); HPRTB, FESFPS, BFXIII (F13B), and LIPOL (Example 4)(Claims 10, 35, 37, 41, 43); HSAC04 (ACTBP2) and CYP19 (Example 5); HSAC04 (ACTBP2), CYP19, and PLA2A1 (Example 6)(Claims 1, 10); HSAC04 (ACTBP2) and FABP (Example 7); APOA2, CYP19, and PLA2A1 (Example 8)(Claims 1, 10); CD4, CSF1PO, and TH01 (Example 9)(Claims 1, 10); CYP19, FABP, and PLA2A1 (Example 10)(Claims 1, 10); CYP19, HPRTB, and PLA2A1 (Example 11)(Claims 1, 10); F13A01 and FABP (Example 12); BFXIII (F13B) and FESFPS (Example 13); BFXIII (F13B), HPRTB, and PLA2A1 (Example 14)(Claims 1, 10); F13A01, FABP, and CD4 (Example

15)(Claims 1, 10); HPRTB and FESFPS (Example 16); HPRTB, FESFPS, and LIPOL (Example 17)(Claims 1, 10); BFXIII (F13B) and LIPOL (Example 18); HPRTB, TPOX, and BFXIII (F13B) (Example 19)(Claims 1, 10); HPRTB, FESFPS, and BFXIII (F13B) (Example 20)(Claims 1, 10); CSF1PO, TPOX, and CD4 (Example 21)(Claims 1, 10); HPRTB, FESFPS, and MYOPK (Myotonic) (Example 22)(Claims 1, 10); CSF1PO, TPOX, TH01, and CD4 (Example 23)(Claims 1, 10, 20, 25); F13A01 and MYOPK (Myotonic) (Example 24); F13A01 and BFXIII (F13B) (Example 25); CSF1PO, TPOX, TH01, and CD4 (Example 26)(Claims 1, 10); CSF1PO, TH01, and CD4 (Example 27)(Claims 1, 10); CSF1PO, TH01, and VWFA31 (Example 28)(Claims 1, 10); HPRTB, BFXIII (F13B), and LIPOL (Example 29)(Claims 1, 10); CSF1PO and TH01 (Example 30); TH01 and CD4 (Example 31)TH01 and TPOX (Example 32).

198. Even ignoring the multiplexes where just two loci were co-amplified, there are seventeen (17) tri-plexes (*i.e.*, where three loci were co-amplified), and three (3) quadruplexes (*i.e.*, where four loci were co-amplified) in the '598 Patent which are not described in the Cited Art. This represents a significant addition to the field "at the time" (*i.e.*, using the Cited Art as representative) and therefore an advancement in STR multiplex PCR. There are many more multiplex combinations, including new combinations, taught in the '598 Patent (along with the associated reaction conditions, primer sequences and the like), as well as observations about how to optimize the assays and overcome the difficulties encountered.

199. In terms of providing explicit protocols (and therefore guidance to anyone who wishes to co-amplify such loci), each of the examples in the '598 Patent describes the particular reaction conditions (discussed more below) used for each of the

multiplexes. Moreover, the '598 Patent describes the sequence of the particular primers used in these reactions. In my opinion, this provides the starting materials and guidance with respect to the direction in which some further experimentation can proceed.

200. In my opinion, any new STR multiplex in 1994 was inventive, even where one added a single new locus to a pre-existing multiplex (*e.g.*, adding a new locus to a multiplex of two loci to make a triplex; adding a new locus to a multiplex of three loci to make a quandruplex, etc.). Moreover, larger STR multiplexes in 1996 (and still larger STR multiplexs in 1998) were inventive, since the difficulty in adding a new locus increases with the size of the pre-existing multiplex. One of the most significant problems in adding new loci is unpredictable interactions between primers. Given this lack of predictability, these multiplexes are not obvious.

201. With regard to primer sequences, the '598 Patent teaches (as do the other Promega patents) that the wrong primers can create serious STR multiplex PCR problems and describes the resulting problems attributable to suboptimal primer design: "Inappropriate selection of primers can produce several undesirable effects such as lack of amplification, amplification at multiple sites, primer dimer formation, undesirable interaction of primer sequences from different loci, production of alleles from one locus which overlap with alleles from another, or the need for amplification conditions or protocols for the different loci which are incompatible in a multiplex." (Promega '598 Patent, col.7, ln 4 – 11; Promega '660 Patent, col.12, ln 66 - col 13, ln 6; Promega '235 Patent, col.11, ln 25 – 32; Promega '771 Patent, col. 11, ln 34-42). This aids the field in recognizing when an STR mPCR reaction could be improved by paying attention to

primer optimization. This is guidance with respect to the direction in which some further experimentation can proceed.

202. This teaching in the '598 Patent is not found in the Kimpton '93 publication which indicates "[m]icrosatellite loci were found to co-amplify with relative ease." (p. 16, left column). There is not even a hint in the Kimpton '93 paper that primer selection can result in multiplex problems. In my opinion, the guidance provided by the '598 Patent regarding the importance of primer sequence reflects an advance in understanding when compared to the Kimpton '93 paper which lacks guidance on the point. The Kimpton '93 paper teaches less than the '598 Patent when it comes to the importance of the particular primers selected.

203. Even where some of the multiplexes in the '598 Patent contain a locus amplified by Kimpton '93, there are often primer sequence differences. For example, Kimpton '93 reports a first primer sequence for the vWA31 locus in Table 1. Example 1 of the '598 Patent reports a first primer sequence for the vWA31 locus which differs from the Kimpton '93 primer sequence by the addition of the bases: GAAAG. The result is a longer primer, which can have different performance characteristics (particularly at higher annealing temperatures, as discussed below).

204. With regard to reaction conditions, there are several steps in the PCR process. First, the "double stranded" or two strands of genomic DNA are separated or "denatured," thereby forming "single stranded" DNA. This denaturation step is done by heating the DNA to a certain temperature. Second, a pair of PCR "primers" is introduced and allowed to hybridize or pair with the single stranded DNA. "Hybridization" occurs when the PCR primers "anneal" or join to a single strand of the DNA. This is also done at

a particular temperature. The third step of the PCR process is extension of the primers that have hybridized to the single stranded DNA molecules by a thermostable DNA polymerase to convert them into double stranded molecules. This is also done at a certain temperature. The temperatures used at each step (and other reaction conditions such as buffers, enzyme concentration, etc.) can be changed in order to change the result of the amplification.

205. The annealing temperatures for the three multiplexes in the Kimpton '93 paper were reported to be 54° C, 60° C and 60° C. Kimpton '93 suggests that changing other reaction conditions simply was not necessary for mPCR of STR loci: "only the annealing temperature requiring adjustment for each specific multiplex system." (p. 16, left column). The '598 Examples describe protocols for amplification involving higher annealing temperatures (e.g. 64° C) and (as noted above) different primer sequences in some instances. One skilled in PCR is thus taught that the longer primer and higher annealing temperature describe in the '598 Patent was used in order to achieve higher specificity in the amplification (*e.g.*, to reduce non-specific amplification and deleterious interactions of primers for different loci).

206. A careful comparison of all of the examples in all of the Promega Patents will reveal other differences (both in terms of combinations of loci, reaction conditions, primer sequences, and the like) teaching the variations that are necessary for successful STR mPCR performance so that one can determine the alleles present at each of the STR loci in the multiplex.

207. Many of the loci used in the multiplexes described in Kimpton '93 and the Caskey patent did not make it through the locus selection process that resulted in what is

known as the CODIS (Combined DNA Index System) database program. That is to say, while loci such as F13AO1, F13B and FES/FPS were considered, they did not end up in the final group of loci selected for the national system by the FBI and other law enforcement bodies. See B. Budowle et al., "CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools," (p. 76) However, many of multiplexes in the '598 Patent contain loci which are all part of the CODIS system, thereby making the multiplex combinations and design of conditions that make them successful taught in the '598 Patent particularly valuable.

208. The '598 Patent teaches one in the field how to go from a specific triplex of CSF1PO-TPOX-TH01 (Example 1) to a specific quadruplex of CSF1PO-TPOX-TH01-vWF (Example 2) by adding a locus, vWF. Kimpton '93 does not provide this teaching, *i.e.* it does not show any examples of how the reaction conditions for a successful triplex can be changed to accommodate an additional locus (thereby making a successful quadruplex from the triplex).[1] Thus, while Kimpton '93 uses some of the same STR loci as the '598 Patent, no guidance is provided for how one could use those loci in the specific STR multiplexes taught in the '598 Patent. A comparison of the details of the two examples in the '598 Patent shows that the primer concentrations had to be adjusted significantly:

	Example 1	Example 2
Locus	Primer Conc.	Primer Conc.
CSF1PO	0.2 μM	1.0 μM
TPOX	0.2 μM	0.15 μM
THO1	0.6 μM	0.2 μM

[1] Each multiplex in Kimpton '93 has a specific set of loci; there are no loci in common.

VWFA31	--------	1.0 μM

209. In addition, the concentration of the polymerase had to be increased. Thus, the Kimpton '93 paper teaches less than the '598 Patent when it comes to providing the technical guidance on how one must adjust the reaction conditions of a multiplex involving some of the same STR loci to make a quadruplex first described in the '598 Patent.

210. Moreover, the changes necessary for adding the locus were determined empirically and the final successful conditions could not be predicted. In fact, Kimpton '93 would have discouraged arriving at this solution because it taught that "all multiplex systems employed identical buffer, dNTP, and enzyme oncentrations." (p.16, left column). Given this lack of predictability, I do not believe these multiplexes are obvious.

b. The 598 Promega Patent Teaches One Skilled in the Art How to Make and Use the Multiplexes Defined in the Asserted Claims of that Patent

211. For the reasons provided below, it is my opinion that the reagents and protocols for successfully multiplexing the sets of loci set forth in the asserted claims of the '598 Patent can be found in the examples.

212. The particular combination of three loci in Example 1 of the '598 patent relate to the same loci specified in independent Claims 12 and 23 of the '598 patent. Claim 15 depends from Claim 12 and adds a locus corresponding to the added locus of Example 2. Claim 24 depends from Claim 23 and adds a locus corresponding to the added locus of Example 2.

213. The second to last triplex listed in independent Claim 1 of the '598 patent (CSF1PO, TH01, and VWFA31) is the same triplex exemplified in Example 28. The second to last triplex listed in independent Claim 10 of the '598 patent (CSF1PO, TH01, and VWFA31) is the same triplex exemplified in Example 28.

214. As noted previously (see paragraph 197 above), the '598 patent has thirty-two (32) examples. The above paragraphs 32 and 33 are merely illustrative of how the examples support the claimed multiplexes.

c. The '660 Promega Patent Contains More than the Cited Art and this Material, Which Includes New Multiplexes, Is Not Obvious in View of the Cited Art

215. By the time the application leading to the '660 Patent was filed in 1996, there had been some advancement in the field and a few multiplex STR assays as large as an octaplex had been achieved. See Promega '660 Patent, col.3, lines 9-12. However, the process of developing new multiplexes was still laborious, unpredictable, and still required extensive trial and error experimentation. Promega '660 Patent, col.12, lines 46-50. The '660 Patent provides guidance as to what properties of new multiplexes should be evaluated in this trial and error process that led to the multitude of STR multiplexes described in this patent. Promega '660 patent, col.13, lines 27-45.

216. The '660 Patent contains thirty-five experimental examples. Examples 26, 28-29, 31 and 34-35 involve multiplexes of three (3) loci. Examples 1-16, 27, 30, and 32-33 involve multiplexes of four (4) loci. Example 17 (6 loci), Example 18 (7 loci), Example 19 (8 loci), Example 20 (6 loci), Example 21 (7 loci), Example 22 (8 loci), and Example 23 (8 loci) involve larger STR multiplexes. This represents a significant

addition to the field and therefore an advancement in STR multiplex PCR. There are many more multiplex combinations, including new and larger combinations, taught in the '660 Patent (along with the associated reaction conditions, primer sequences and the like), as well as observations about how to optimize the assays and overcome the difficulties encountered. The Cited Art does not teach the multiplexes of the '660 Patent. Moreover, since these combinations were new and the conditions for getting them to work were determined empirically and were not predictable, I do not believe that these muliplexes are obvious.

217. In terms of providing explicit protocols (and therefore guidance to anyone who wishes to co-amplify these loci), each of the examples in the '660 Patent describes the particular reaction conditions used for each of the multiplexes defined in the asserted claims of the '660 patent. Moreover, the '660 Patent describes the sequences of the particular primers used in these multiplexes.

218. Many of multiplexes in the '660 Patent contain loci which are all part of the CODIS system, thereby making the multiplex combinations and design of conditions that make them successful taught in the '660 Patent particularly valuable.

219. The '660 Patent teaches one in the field how to go from a triplex of CODIS loci (Example 26) to a quadruplex of CODIS loci (Example 15) by adding another CODIS locus. The '660 Patent teaches one in the field how to go from a key CODIS quadruplex (Example 15) to larger multiplexes involving CODIS loci (Examples 17, 18 and 19) by adding loci. As noted previously, the Cited Art does not provide such a teaching for any STR multiplex.

220. A comparison of the reaction conditions between examples reveals changes in the primer concentrations as well as polymerase concentrations that were necessary to achieve a hexaplex reaction containing the same 4 STRs as the smaller quadruplex:

	Example 15	Example 17
Taq	0.04 U	0.06 U
D16S539	0.60 µM (2-FL)	0.65 µM (2-FL)
D7S820	0.325 µM (2-FL)	0.325 µM (2-FL)
D13S317	0.22 µM (2-FL)	0.22 µM (2-FL)
D5S818	0.50 µM (2-FL)	0.55 µM (2-FL)
HUMCSF1PO	----------	0.40 µM (1-TMR)
HUMTPOX	----------	0.40 µM (2-TMR)
HUMTH01	----------	----------
HUMvWFA31	----------	----------

221. As discussed above, Kimpton '93 would have discouraged arriving at this solution because it taught that "all multiplex systems employed identical buffer, dNTP, and enzyme concentrations." (p.16, left column).

d. The '660 Promega Patent Teaches One Skilled in the Art How to Make and Use the Multiplexes Defined in the Asserted Claims of that Patent

222. For the reasons provided below, it is my opinion that the reagents and protocols for multiplexing the sets of loci set forth in claims 2-5, 16-17, 19-21, 23-25 and 27-31 of the '660 Patent can be found in the examples.

223. The triplex of Example 26 corresponds to the second to last triplex specified in Claim 16 and the fifth listed triplex of Claim 25. The quadruplex of CODIS

loci in Example 15 involve the same loci specified in the last set of loci of Claim 2 of the '660 Patent and the last quadruplex listed in Claim 25.

224. The six loci of Example 17 involve the same loci specified in the first set of loci of Claim 3 of the '660 Patent. The six loci of Example 20 involve the same loci specified in the second set of loci of Claim 3 of the '660 Patent.

225. The seven loci of Example 18 involve the same loci specified in the first set of loci of Claim 4 of the '660 Patent. Thus, Example 18 provides an illustration of adding a locus to the first set of six loci of Claim 3 of the '660 Patent to achieve the larger claimed multiplex.

226. The seven loci of Example 21 involve the same loci specified in the second set of loci of Claim 4 of the '660 Patent. Thus, Example 21 provides an illustration of adding a locus to the second set of six loci of Claim 3 of the '660 Patent to achieve the larger claimed multiplex.

227. The eight loci of Examples 19 and 23 involve the same loci specified in the first set of loci of Claim 5 of the '660 Patent. Thus, Examples 19 and 23 provide an illustration of adding a locus to the first set of seven loci of Claim 4 of the '660 Patent to achieve the larger claimed multiplex.

228. The eight loci of Example 22 involve the same loci specified in the second set of loci of Claim 5 of the '660 Patent. Thus, Example 22 provides an illustration of adding a locus to the second set of seven loci of Claim 4 of the '660 Patent to achieve the larger claimed multiplex.

229. Table 1 of the '660 Patent provides Sequence Identification numbers (SEQ ID) for primer pairs for certain loci. Table 2 of the '660 Patent introduces, in some cases,

new primers for the same loci. Thus, a variety of starting materials are provided in the '660 Patent.

e. The '235 Promega Patent Contains More than the Cited Art and this Material, Which Includes New Multiplexes, Is Not Obvious in View of the Cited Art

230. By the time the application leading to the '235 Patent was filed in 1998, there had been some further advancement in the field and "systems containing up to 11 separate STR loci" had been described. *See* Promega '235 Patent, col. 3, lines 6-8. However, the process of developing new multiplexes was still laborious, unpredictable, and still required extensive trial and error experimentation. *See* Promega '235 Patent, col. 9, lines 6-7. The '235 Patent provides guidance as to what properties of new multiplexes should be evaluated in this trial and error process. Indeed, the body of the specification focuses on the additional parameters and indications of success and failure that the inventors had observed in their work and which are useful in this type of trial and error experimentation necessary to develop multiplex STR PCR reactions.

231. The '235 Patent contains six experimental examples. Example 1 involves multiplexing 13 STR loci: D3S1358. HUMTH01, D21S11, D18S51, HUMvWFA31 (also known as vWA), D8S1179, HUMTPOX, HUMFIBRA (also known as FGA), D5S818, D7S820, D13S317, D16S539, and HUMCSF1PO. These are all of the CODIS loci. To achieve this, twenty-six (26) primers were used in combination in a single reaction vessel. Example 1 provides the reaction conditions and primer sequences for performing this large multiplex. The results of the amplification were evaluated on an ABI PRISM machine using capillary electrophoresis. A three color labeling system was used. As a

result, overlap of alleles from different loci was not a problem. The results are shown in Figures 1A (Panels A, B and C) and 1B (control).

232. Since I consider the achievement of a multiplex of all CODIS loci to be a significant advancement in the field, I do not believe such multiplexes are obvious. These combinations were new and the conditions for getting them to work were determined empirically and were not predictable.

233. Example 2 of the '235 Patent amplifies still more loci in a multiplex: D3S1358, HUMTH01, D21S11, D18S51, G475, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 (plus the Amelogenin marker). This represents all of the CODIS loci plus the G475 and S159 loci (which were loci not previously known in the literature). To amplify these fifteen (15) STR loci and the Amelogenin marker, thirty-two primers were used in combination in a single reaction vessel. Example 2 of the '235 Patent provides the reaction conditions and primer sequences for this large multiplex. The results of the amplification were evaluated on an ABI PRISM instrument using capillary electrophoresis. Again, a three color labeling system was used. As a result, overlap of alleles from different loci was not a problem. The results are shown in Figures 2A (Panels A, B and C) and 2B (control).

234. Example 3 of the '235 Patent amplifies the same loci as in Example 2: D3S1358, HUMTH01, D21S11, D18S51, G475, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO, and S159 (plus the Amelogenin marker). The Example indicates the same reaction conditions and primer sequences were used as in Example 2. However, the analysis was carried out on a different instrument. Again, a three color labeling system was used. As a result, overlap

of alleles from different loci was not a problem. The results are shown in Figures 3A (Panels A, B and C) and 3B (control).

235. Example 4 of the '235 Patent describes more than one locus combination for amplification. A first locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA (*i.e.*, nine STR loci plus the Amelogenin marker). A second locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159. A third locus combination included the following: D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159. Example 4 of the '235 Patent provides the reaction conditions and primer sequences for each of these multiplexes. The concentration of the polymerase was different for each combination. The results were separated on a 4% denaturing polyacrylamide gel and evalulated on a Hitachi instrument. In this case, a two color labeling system was used. As a result, overlap of alleles from different loci was not a problem. The results are shown in Figures 4A and 4B.

236. Example 5 of the '235 Patent describes more than one locus combination for amplification. A first locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. A second locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and C221. A third locus combination included the following: D5S818, D7S820, D13S317,

D16S539, HUMCSF1PO and C221. Example 5 of the ‘235 Patent provides the reaction conditions and primer sequences for each of these multiplexes. The concentration of the polymerase was different for each combination. The results were evalulated on a Hitachi instrument. In this case, a two color labeling system was used. As a result, overlap of alleles from different loci was not a problem. The results are shown in Figures 6A and 6B.

237. Example 6 of the ‘235 Patent describes more than one locus combination for amplification. A first locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, and HUMFIBRA. A second locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, G475, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159. Example 6 of the ‘235 Patent provides the reaction conditions and primer sequences for each of these multiplexes. The concentration of the polymerase was different for each combination. The results were evalulated on a Hitachi instrument. In this case, a two color labeling system was used. As a result, overlap of alleles from different loci was not a problem. The results are shown in Figures 5A and 5B.

238. The Cited Art does not disclose or teach the many multiplexes of Examples 1-6 of the ‘235 patent. These combinations were new and the conditions for getting them to work were not taught by, nor obvious in view of, the Cited Art.

f. The '235 Promega Patent Teaches One Skilled in the Art How to Make and Use the Multiplexes Defined in the Asserted Claims of that Patent

239. For the reasons provided below, it is my opinion that the reagents and protocols for multiplex PCR of the loci set forth in the claims of the '235 Patent can be found in the examples.

240. Claim 1 of the '235 Patent specifies the following loci in a multiplex: D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31. As noted above, Example 1 of the '235 Patent describes the experimental work for a multiplex that includes the following loci: D3S1358. HUMTH01, D21S11, D18S51, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, and HUMCSF1PO. Since these are the same loci, Example 1 corresponds to Claim 1 of the '235 Patent.

241. Claim 13 of the '235 Patent specifies the following loci in the multiplex: D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, and HUMvWFA31. As noted above, Example 1 provides the experimental work for a multiplex having these same loci.

242. Claim 18 of the '235 Patent specifies primers for the following loci in the kit: D3S1358, D5S818, D7S820, D8S1179, D1S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, AND HUMvWFA31. As noted above, Example 1 provides the experimental work for a multiplex having these same loci.

243. Tables 1 and 2 of the ‘235 Patent provide more than one primer pair per locus. Some of these primers are different in sequence and length. Thus, a variety of starting materials are supplied for developing multiplexes for these loci.

244. The task of developing a new set of primers for a locus already present in an established multiplex (*e.g.*, to improve or optimize performance) should not be equated (in the degree of difficulty or complexity) with the challenge of adding a completely new locus. When one has an established multiplex, one knows a set of conditions in which a particular set of primers are successful. Therefore, in designing new primers, one has a lot of data as to what works with that particular set of loci, and this data can guide primer design. For example, one need not try a lot of alternative conditions, given that one knows of one set that works with some primers. One knows a lot about the characteristics of the primers that worked, such as their melting temperature (T_M), GC content, clustering of AT or GC sequences, nature of the sequences at the 3’ end, and the like. This is very helpful in designing new primers. One of the simplest modifications is moving the size range of amplicons a few nucleotides. One can usually be successful by leaving the 3’ end alone and simply extend the 5’ end of the primers.

g. The ‘771 Promega Patent Contains More than the Cited Art and this Material, Which Includes New Multiplexes, Is Not Obvious in View of the Cited Art

245. The ‘771 Patent has the same specification as the ‘235 Patent (which is discussed above). Thus, the ‘771 Patent, like the '235 Patent, provides guidance as to what properties of new multiplexes should be evaluated in this trial and error process. Indeed, the body of the specification focuses on the additional parameters and indications of success and failure that the inventors had observed in their work and which are useful in this type of trial and error experimentation necessary to develop multiplex STR PCR reactions.

246. The ‘771 Patent contains the same six experimental examples as the ‘235 Patent. The Cited Art does not disclose or teach the many multiplexes of Examples 1-6 of the ‘235 patent. These combinations were new and the conditions for getting them to work were not taught by, nor obvious in view of, the Cited Art.

h. The ‘771 Promega Patent Teaches One Skilled in the Art How to Make and Use the Multiplexes Defined in the Asserted Claims of that Patent

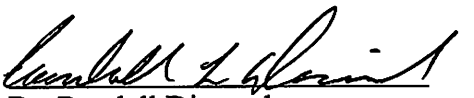
247. For the reasons provided below, it is my opinion that the reagents and protocols for multiplex PCR of the loci set forth in the claims of the ‘771 Patent can be found in the examples.

248. Claim 5 of the ‘771 Patent specifies primers for the following loci in the kit: D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, HUMCSF1PO, HUMFIBRA, HUMTH01, HUMTPOX, HUMvWFA31, and a locus

selected from the group consisting of G475, S159, C221, and Amelogenin. Example 2 of the '771 Patent amplifies the following loci in a multiplex: D3S1358, HUMTH01, D21S11, D18S51, G475, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and S159 (plus the Amelogenin marker). This represents all of the 13 CODIS loci plus G475, S159 and Amelogenin. Example 5 provides the experimental work for a second locus combination included the following: D3S1358, HUMTH01, D21S11, D18S51, S159, Amelogenin, HUMvWFA31, D8S1179, HUMTPOX, HUMFIBRA, D5S818, D7S820, D13S317, D16S539, HUMCSF1PO and C221. Thus, Claim 5 of the '771 Patent specifies loci which are exemplified in the experimental work of the specification.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on September 1, 2011.



Dr. Randall Dimond